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A Summary of Current Program, 7/1/63

and Preliminary Report of Progress

for 7/1/62 to 6/30/63

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UNITED STATES DEPARTMENT OF AGRICULTURE

Section B

This progress report of U.S.D.A. and cooperative research is primarily a tool for use of scientists and administrators in program coordination, development and evaluation; and for use of advisory committees in program review and development of recommendations for future research programs.

The summaries of progress on U.S.D.A. and cooperative research include some tentative results that have not been tested sufficiently to justify general release. Such findings, when adequately confirmed will be released promptly through established channels. Because of this, the report is not intended for publication and should not be referred to in literature citations. Copies are distributed only to members of Department staff, advisory committee members and others having a special interest in the development of public agricultural research programs.

This report also includes a list of publications reporting results of U.S.D.A. and cooperative research issued between July 1, 1962 and June 30, 1963. Current agricultural research findings are also published in the monthly U.S.D.A. publication, Agricultural Research. This progress report was compiled in the Entomology Research Division, Agricultural Research Service, U. S. Department of Agriculture, Beltsville, Md.

UNITED STATES DEPARTMENT OF AGRICULTURE
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TABLE OF CONTENTS

Section B

		Page
Area No. 13	Beef Cattle, Horse, and Swine Insects	169
Area No. 14	Dairy Cattle Insects	195
Area No. 15	Sheep and Goat Insects	220
Area No. 16	Poultry Insects	231
Area No. 17	Insects Affecting Man, Households, and Industrial Establishments	242
Area No. 18	Bees and Other Pollinating Insects	258
Area No. 19	Analysis, Synthesis, Formulation, and Evaluation of Insect Control Chemicals	275
Area No. 20	Identification of Insects and Related Arthropods	290
Area No. 21	Foreign Exploration, Introduction, and Evaluation of Biological Control Agents	298
Area No. 22	Insect Pathology	306
Area No. 23	Insect Physiology and Mode of Action of Insecticides and Their Metabolites	317
Line Project	Check List	327

AREA NO. 13. BEEF CATTLE, HORSE, AND SWINE INSECTS

Problem. Insects and ticks irritate and torment livestock throughout the year in all parts of the United States and cause serious losses. These pests reduce weight gains, lower the quality of meat and hides, and spread numerous animal diseases. Livestock losses directly attributable to insects and ticks are estimated to exceed $300 million annually. Practical but not adequate control methods have been developed for lice, screw-worms, ticks, bots, grubs, and other insects, but satisfactory methods of protecting cattle from horse flies, deer flies, stable flies, mosquitoes, and the newly introduced face fly remain an unsolved problem. The development of insecticides for use on beef cattle, horses, and swine has been hampered because certain insects have become resistant to various insecticides, and because harmful residues have been found in meat following the application of certain materials. Safe, effective, nonresidue-forming insecticides and repellents are required. Urgently needed are economical and long-lasting insecticides or repellents for range cattle to protect them against vicious biting flies. Safer, cheaper, and more effective systemic insecticides and more efficient means of administration are needed to combat grubs and bots in cattle and horses. New approaches to control, including radiation and chemosterilants, should be explored to determine their feasibility as practical control methods. Efforts also need to be made to find and evaluate insect pathogens, parasites, and predators for controlling certain livestock pests. Expanded basic studies on the biology and physiology of these pests are needed to find weak links in their life cycles that will serve as a basis for the development of more effective and safer methods of control. Research also is urgently needed on the role of insects in the spread of diseases of livestock.

USDA PROGRAM

The Department has a continuing, long-term program involving basic and applied research on insects and ticks which affect the health and productivity of beef cattle, horses, and swine. Studies are conducted on the biology, physiology, genetics, and nutrition of the screw-worm, stable fly, horn fly, house fly, mosquitoes and other pests; on the nature of insect resistance to insecticides; and on absorption, metabolism and excretion of insecticides by insects feeding on or in animals; the effects of irradiation and chemosterilants on insects; insect attractants and repellents; and other new approaches to control. Research is concerned with the development of more effective contact and systemic insecticides and protective treatments for the control of livestock pests. Studies are conducted to determine the occurrence of residues in tissues of animals treated with insecticides. Minor consideration is given to the development of sanitation and management procedures and biological control methods, including parasites and predators, for controlling the face fly, stable fly and several other pests. Emphasis is also given to the development of insect sterility, attractants and

various other noninsecticidal approaches to control. Studies are conducted in cooperation with the Agricultural Engineering and Animal Husbandry Research Divisions to evaluate various kinds of traps and devices for estimating and controlling natural insect populations, and improved or special equipment for the application of insecticides to animals. Limited research is conducted on the role of insects and ticks as vectors of livestock diseases, with particular emphasis on bovine anaplasmosis and equine piroplasmosis. The Federal scientific effort devoted to research in this area totals 16.3 professional man-years. Of this number, 6.1 is devoted to basic biology, physiology and nutrition; 3.9 to insecticidal and sanitation control; 2.0 to insecticide residue determinations; 0.2 to biological control; 2.0 to insect sterility, attractants and other new approaches to control; 0.2 to the evaluation of equipment; 0.8 to insect vectors of diseases; and 1.1 to program leadership.

REPORT OF PROGRESS FOR USDA AND COOPERATIVE PROGRAMS

A. Basic Biology, Physiology and Nutrition

1. Mosquitoes. At Corvallis, Oreg., and Fresno, Calif., studies were continued on the biology and ecology of important mosquito species. In laboratory tests the majority of Culex tarsalis females preferred to feed at the higher of two feeding locations and preferred mice rather than chickens for their source of blood.

A study was conducted on the daily activity of several species of mosquitoes --Anopheles freeborni, Culex peus, Culiseta inornata, Culex incidens, and Culex tarsalis. All species behaved similarly. Both males and females began to move out of daytime resting shelters a few minutes before or after sunset. Only a few males and females (0.3 to 2.8%) remained in the shelters during the night. Adults did not begin to return to the shelters until sunrise or a few minutes later, and all did not return until sometime after 8:00 a.m. Exodus from and return to shelters appeared to be regulated by light intensity. Other factors such as preconditioning to light cycles, temperature, and humidity, may have had some effect since artificial lights did not prevent mosquitoes from leaving shelters eventually, but did change their time and rate of leaving. Use of special crosses of resistant and susceptible strains of Culex tarsalis showed that some of the females mate more than one time and utilize sperm from more than one mating.

Taxonomic studies defined suitable characters for separating dark-winged forms of Aedes dorsalis from A. melanimon and determined that these two forms coexist in only one area (Solano County, Calif.). Surveys at Borrego Springs (San Diego County, Calif.) were made to find isolated populations of mosquitoes for future studies on control through sterilization.

Surveys were continued on biting arthropods of the Humboldt River Basin in Nevada. During April, May, and June, water was plentiful and high populations of Aedes melanimon, A. dorsalis, A. vexans, Culex tarsalis, and Culiseta inornata were found. Mosquito larvae were found infected with a

microsporidian, Thelohania sp. and a bacterium, both of which are under study as biological control agents. In late July, no floodwater Aedes breeding was observed, but moderate breeding of Culex tarsalis and Culiseta inornata was observed. Studies of mosquito larval populations in typical breeding areas in dairy drains and culverts in California during April indicated the presence of Culiseta inornata, Culiseta incidens, Culex tarsalis, Culex peus, Culex apicalis, and Anopheles franciscanus. No breeding of Culex quinquefasciatus was found.

In Oregon, using C-14 labeled TDE, slight but consistent differences in the rate of degradation of TDE have been noted in susceptible and resistant strains of Culex tarsalis, indicating that the main source of resistance to this chemical is increased degradation of TDE to FW-152 and water soluble metabolites through an oxidative rather than a dehydrochlorination mechanism. Further tests with DDT and TDE have shown that analogs of DDT, which block metabolism via the oxidative pathway, overcome resistance to DDT and tend to confirm the hypothesis that resistance to DDT and TDE in tarsalis is due to a more rapid oxidative metabolism of the insecticide.

Early in July 1962, reports were received of a serious mosquito outbreak in the cattle-growing areas along the Gulf Coast of Louisiana and Texas. Surveys in these areas revealed a heavy mosquito population resulting from successive broods of mosquitoes over a period of 6-8 weeks. At the time of the survey, the spring calf crop had been decimated and hundreds of mature cattle had perished from the attacks of mosquitoes. Growers had sold or moved thousands of animals to avoid further losses. The loss incurred by cattlemen was estimated at $250,000. Because of the large area involved, control of the mosquitoes was not feasible.

2. Stable Fly. In Texas stable flies began to feed when they were only 4 hours old. The adults did not feed well or survive long on deer blood or several synthetic diets. They thrived on beef blood and beef serum but not on blood cells alone. At 80°F. and 50-60% relative humidity first matings occurred when the flies were 3 days old and 84% mated within 5 days. At 80°F. and 10-30% relative humidity mating did not begin until flies were 4 days old and only 12% mated in 5 days. No mating whatever occurred at 68°F. and uncontrolled humidity.

Stable fly larvae were not adversely affected by 17 hours of submergence in water, but 100% mortality resulted from 24 hours submergence. Exposure of pupae in water for 17 hours resulted in retarded development and greatly reduced adult emergence.

In Oregon field studies determined the dispersal pattern of marked adult stable flies. Flies were recovered over 14 miles from the point of release in 24-48 hours.

3. Face Fly. In Nebraska, face flies were first observed on cattle in the field on April 25 and by mid May they were present in annoying numbers. By early June heavy populations of 20 or more per head were widespread.

Studies on the behavior of the face fly were conducted in a large outdoor screened cage in which a calf was restrained. Several hundred laboratory-reared flies were released in the cage before each observation period. Less than 4% of the flies were present on the calf at any one time. The remainder rested in the grass or on the sides of the cage. Only an occasional male was observed on the calf. Nearly half of the flies congregated around the eyes and about 25% on the muzzle.

4. Horn Fly. In Texas, after considerable trial and error, a technique was developed for colonizing the horn fly in the laboratory without access to a bovine host. The diet consisted of 1 part bovine blood and 1 part beef juice plus 1 mg. of streptomycin, 1000 units of penicillin and 250 units of mycostatin per ml. of diet. Flies were fed by placing a cotton pad soaked with the diet on top of the holding cages. Cages consisted of 4" lengths of 6" plastic tubing with ends of plastic screen. Eggs were deposited on the plastic screen. Optimum conditions for adult reproduction were 90°F. temperature and 60-80% relative humidity. The colonization procedure is easy and inexpensive and greatly facilitates laboratory testing of insecticides, chemosterilants and repellents at all seasons of the year. Adult flies survived for 96 hours in a room kept at 75-80°F. and 62-67% relative humidity. Only a few survived in a room kept at 85-92°F. and 34-40% relative humidity.

In Oregon field studies determined the pattern of dispersal of marked adult horn flies. Flies were recovered over 7 miles from the point of release in 24-48 hours.

5. Screw-worm. Research on the screw-worm was discontinued at Kerrville, Tex., in September 1962 and moved to Mission, Tex., headquarters of the Southwest eradication campaign. This included research on the biology and ecology of the screw-worm under field conditions, vigor and longevity of sterilized flies, effects of radiation, the development of genetically marked strains, cytological studies with irradiated flies, nutrition, and special tests related to problems in the sterile male release program.

A new technique to determine the mating aggressiveness of sterilized or mutant screw-worm flies was developed. It was observed that in laboratory cages males harassed females sufficiently to cause mortality greater than that occurring when females were caged without males--in general, higher ratios of males to females caused greater mortality than lower ratios. Tests with various strains of screw-worms of known sexual competetiveness in comparison with other mating aggressiveness tests confirmed the validity of the method. Since the criterion for mating aggressiveness is the mortality of females over time rather than egg production and viability, the time, space, and labor required to determine sexual aggressiveness were

greatly reduced. Studies confirmed optimum numbers of screw-worm larvae per tray for rearing efficiency and optimum numbers of adults per cage for mating and longevity studies. They also confirmed diurnal periodicity in screw-worm pupae for emergence occurred from sunrise until noon. Studies indicated that the use of CO_2 as anesthesia for screw-worms is safe for handling young screw-worm adults for longevity and mating tests and scanning for genetic markers.

Research was continued to find and develop genetically distinct strains of screw-worm flies and to study these mutant flies. In the adults many genetic markers were found such as yellow eyes and white auxillary region ("Whaxy"). The genetic marker "Whaxy" affects the morphology of both the adult and larvae--a factor which would be invaluable in field studies. Approximately 270,000 screw-worm larvae were examined for morphological variants and from this 221 cultures were studied as possible genetic strains. Of markers found in the immature forms, at present three morphological characters--interrupted bands of segmental spines, two spiracular openings in the anal plates of third-instar larvae, and additional spines on the eleventh anterior segmental band--were demonstrated to be genetic in nature. None of these were established as a pure strain. Research with these and other mutant strains is being pursued to determine the genetics of the strains and if these strains are as vigorous and aggressive as the normal strain, factors which are essential if the strains are to be used in the release program. For example, preliminary genetic studies indicate that the "Whaxy" mutant is lethal in the homozygous state with only a few individuals escaping. Other strains have slight behavioral differences from the normal strain, i. e., they respond differently to attractants and survey traps.

When pupae of the screw-worm were irradiated in an atmosphere of CO_2 and air (50-50 mixture), damage to the reproductive system of the adult female was greater than that induced by a similar radiation treatment in air. When the pupae were pretreated in the gas mixture for approximately 45 minutes, complete sterility was induced by a treatment of 4,500 r delivered in CO_2 and air, whereas irradiation in air alone required about 5,500 to 6,200 r. Techniques were developed to study the cytological effects of radiation and chemosterilants on screw-worm, particularly on spermatogenesis and oogenesis. Preliminary studies revealed chromosomal aberrations severe enough to cause dominant lethality. Considerable data on oogenesis and spermatogenesis in normal screw-worm flies were accumulated as necessary background data for evaluating the effects of radiation and chemosterilants on reproduction.

For rearing larvae of the screw-worm, a synthetic diet was developed that contained casein, yeast extract, cholesterol, inorganic salts, water, and agar. This medium was further defined by replacement of casein with a mixture of L-isomer amino acids, and of yeast extract with a mixture of B-vitamin and RNA. Larval growth and development on the defined medium were nearly equal to that on media containing casein and yeast. Larvae

absolutely required thiamine, riboflavin, panthothenate, niacin, and choline for growth. Biotin and folic acid stimulated growth and were necessary for maturation to the adult stage. Pyridoxine and its analogs, pyridoxal and pyridoxamine, inositol, B_{12}, and carnitine had no effect on growth. Niacinamide spared niacin, but p- aminobenzoic acid had no effect on the folic acid requirement.

Studies on the effect of dessication, lack of adult food, reduced temperature, age of flies at time of release, and the effect of all of these factors on different strains of the screw-worm were undertaken to improve the survival of screw-worm flies released into the hot and dry climate of Texas. Provision of food in release cartons or the judicious use of reduced holding temperatures curtailed mortality prior to or shortly after release. Techniques need to be developed which would allow greater uniformity in age of flies at the time of release. Newly emerged flies carry a food and water reserve that will sustain them up to 24 hours (90°F. and 30% R.H.) compared to only 3 to 4 hours for 2-day-old flies. Selection of flies for individuals more capable of survival under unfavorable conditions has shown promising results.

Extensive studies were made of the ecology of screw-worm flies under field conditions by releasing tagged flies. It was determined that flies tend to congregate and disperse along water courses or streams and are capable of traveling long distances. This research resulted in the addition of stretegic releases of screw-worms along water courses and an increase in the effectiveness of the sterile-male release program.

6. House flies. At Orlando, Fla., research on control or eradication through the use of sterilization by radiation or chemicals has shown that considerable gaps exist in knowledge of the biology and mating behavior of house flies. Mating tests have shown that both males and females must undergo a sexual maturation time of at least 16 hours with males and a minimum of 24 hours with females. Once the mating drive has started, males will attempt to "strike" or mate with both males and females and certain inanimate objects, although they "strike" more readily and frequently with females. This fact along with experiments in a large cage-type chemotactometer suggested the presence of some type of a female sex attractant of a low order. Imperfect sex recognition in the male combined with a low order sex attractant in the female would account for the fact that males attempt to mate with either sex and result in the higher ratio of male-to-female "strikes." Quantitative data on the male and female mating behavior and a detailed description of the mating "strike" of the male and mating behavior of the male and female have been developed. Males or females which had their wings removed were able to mate with individuals of the opposite sex with wings. Amputation of more than one pair of legs from the male prevented mating, while amputation of only one pair of legs impeded but did not prevent mating.

Tests were run to determine the actual time house flies remain in copula after initial seizure. Of 61 mating pairs that were trapped and observed, the shortest mating period was 44 minutes and longest, 96 minutes. The average was 60 minutes. Females examined after only 1 and 2 minutes of mating contained no sperm. Those examined after 3 to 5 minutes of mating had spermathecae which contained a few sperm and some which were completely filled. After mating for periods of 10 to 76 minutes, the spermathecae were filled to capacity with sperm, with only one exception.

Eighty-six female house flies reared in the laboratory laid an average of 94.4 eggs per female of which 89% hatched. The number of eggs laid varied from 12 to 186 per female. Under laboratory conditions some flies fed within an hour after emergence; others did not feed until later and some did not feed until they were 16 to 18 hours old.

At Corvallis, Oreg., studies were continued on the physiology of resistant and susceptible house flies. Isolan-resistant house flies have decreased ali-esterase activity and increased Isolan-detoxifying enzyme. Techniques using high-speed centrifugation have been developed which concentrate enzymes important in metabolizing or conferring resistance to organophosphorus compounds. In the case of malathion-resistant house flies, the nature of the alkyl groups attached directly to the phosphorus atom is the major factor in resistance. Thus, the primary cause of resistance is probably associated with the rate of recovery of the phosphorylated detoxifying enzyme after poisoning, rather than with an increased ability to cleave the toxic molecule per se.

In Oregon extensive studies were conducted on the biology, nutrition, and colonization of the little house fly (Fannia canicularis). After much trial and error a satisfactory rearing medium was developed which consisted of alfalfa meal, yeast extract solution and wood shavings. Adult flies were held in standard cages and provided with wrinkled balls of black muslin impregnated with alfalfa and yeast extracts for oviposition sites. Under such conditions 48% of the females and 25% of the males survived for 22 days in cages kept at 70-80°F. At 90°F. no males and only 8% of the females survived for 22 days. Egg deposition was about 5 times as great at 80°F. as at 70° or 90°F. Females began ovipositing in 5-6 days at 90°F., 8-9 days at 80°F. and 10-11 days at 70°F. The eggs dessicate rapidly and must be kept constantly on a moist surface to insure a high percentage of hatch.

7. Cattle Grubs. Research was continued in Texas and Oregon to determine the nutrition requirements of cattle grub larvae and to develop an artificial medium for rearing larvae under laboratory conditions. In Texas various methods and media were tried in an effort to rear grub larvae. First stage larvae survived as long as 13-15 days in several tests but no second and third instars survived more than a week.

In Oregon, studies were conducted to determine the optimum conditions for larval pupation and development. Pupae held in moist sand at 40°, 44° and 50°F. produced no adults. Some emergence occurred at constant temperatures of 60° and 68°F. and at fluctuating temperatures ranging from 63°-75°F. Emergence was greater under fluctuating temperatures than at constant temperatures. Examination of pupae indicated that failure of adults to emerge was due to inability to eclose.

8. Horse Flies and Deer Flies. Studies were continued in Mississippi on the biology of tabanids. First adults of Hybomitra lasiophthalmus were noted on March 28. By mid-April this species was abundant and small numbers of T. vittiger schwardti and T. fusciacostatus were present. The usual succession of species appeared during the summer and early fall months but none reached their usual abundance. Population declined drastically during the latter part of July as a result of prolonged drouth. Populations rose only slightly following rains in late August.

9. Lice. Studies in Oregon showed that low louse populations on cattle during the summer months are largely due to destruction from licking. Short summer hair coats do not protect the lice, whereas long winter coats do. When short coated animals were restrained from licking themselves, heavy louse populations developed in 3-4 weeks.

10. Ticks. Observations in Texas showed that the lone star tick became active in January 1962, and population on cattle increased gradually during February, March and April, reaching a peak in May and June. Tick populations began to decline in July and had virtually disappeared by September.

The winter tick first appeared on cattle in October 1962, and populations gradually increased to a peak in December 1962, and January 1963. Tick populations declined rapidly in February and had virtually disappeared by late March.

B. Insecticidal and Sanitation Control

1. Mosquitoes. Studies were continued at Orlando, Fla., to find new and effective insecticides for the control of mosquitoes. In screening tests with Anopheles quadrimaculatus larvae, 28 of 81 compounds tested were rated Class IV in effectiveness. Four of these compounds--American Cyanamid CL-18133, Stauffer B-10046, Stauffer 8-10094, and Bayer 47940--were highly effective, killing 100% of the larvae at concentrations of 0.05 to 0.01 p.p.m. One hundred and nine plant extracts were also screened for toxicity, but none of these were toxic at low concentrations. Of 83 compounds tested against female Aedes taeniorhynchus in wind tunnel tests, 7 were as effective as the standard, malathion. Dimethrin in granular formulations was effective in laboratory and field tests as a larvicide against Anopheles quadrimaculatus, Aedes aegypti, Aedes taeniorhynchus and Culex quinquefasciatus mosquitoes. A mixture of DDT and an anti-resistant compound was no more effective than DDT alone against C. quinquefasciatus larvae.

In Oregon, studies were continued to find repellents for protecting animals from attacks by mosquitoes. Only one (ENT-26455) of 91 compounds tested was a highly effective repellent. This material showed 100% repellency for 24 hours and from 50 to 90% repellency after 48 hours. Several materials were highly effective toxicants but none was superior to the standard, Bayer 29493.

In Oregon, 46 compounds were tested for systemic action by giving them orally to mice and allowing mosquitoes to feed on the mice. At a dosage of 100 mg./kg., two compounds--Bayer 29493 and Shell SD 8436--killed all mosquitoes for 6 hours after the mice had been treated.

2. Stable Fly. In Texas, 240 compounds were screened in spot tests on cattle for repellency and toxicity against the stable fly. Of these materials, 6 were Class IV repellents at 5% and 21 were Class IV toxicants at 0.5%. A number of other materials were Class IV at higher concentrations. The outstanding repellents were ENT-20274, ENT-25927, ENT-26864, ENT-32965, ENT-25946, and ENT-27031.

Tests were conducted with the WHO test kit to determine the susceptibility of non-resistant stable flies to DDT and dieldrin. At an exposure of 30 minutes the LD-50s and LD-90s for DDT were 1.7 and 2.6% and for dieldrin 0.36 and 0.91%, respectively.

In field tests in Nebraska, applications of 1 pint of 0.5% Ciodrin to cattle gave good control of stable flies for 3-4 days. Daily applications with an automatic sprayer of 100 cc of 0.8% Ciodrin maintained very good control of stable flies. In field tests in Texas, sprays of 0.25% DOWCO 175 (ENT-25964) applied at the rate of 2 quarts per animal gave excellent control of stable flies for 3 days.

3. Face Fly. In Nebraska, tests were conducted in which materials were administered to cattle in feed in order to determine their effectiveness in preventing fly breeding in feces. Concentrations of 0.1% and higher of Bacillus thuringensis, an insect pathogen, prevented fly larval breeding in droppings. At concentrations of less than 0.1% larval survival increased in proportion to the decrease in concentration. Phenothiazine (2.5-3 microns) administered at the rate of 2 grams daily for 12 days gave erratic results but in no instance provided effective control of larval breeding. A concentration of 20 p.p.m. of Co-ral in feed completely inhibited larval breeding and DDVP at 0.5 mg./kg. was almost completely effective. Free choice consumption of salt containing 0.5 or 1.0% Co-ral gave erratic results, larval control ranging from 40 to 85%. The erratic results were attributed to differences in consumption (0.06 to 0.35 mg./kg. daily) by individual animals.

In Nebraska, tests were conducted in which 4% malathion and 0.8% pyrethrins plus 1.1% piperonyl butoxide were installed around salt boxes. Daily contact with the dust bags caused reduction in face fly populations on cattle.

4. Horn Fly. Extensive field tests were conducted in Texas, Mississippi, Nebraska, and Oregon, to compare several old and promising new insecticides for the control of horn flies on cattle. In Texas sprays of 0.25 and 0.5% Hooker 1422 (ENT-25780) applied at the rate of 2 quarts per animal provided effective control of horn flies for 7-10 days. Applications of 2 quarts of 0.5% Ciodrin and 1 pint of 2% Ciodrin provided 14-95 days control in dry central Texas and 11-12 days control in humid east Texas. Tests were conducted with several materials applied in minimal amounts by an automatic mist sprayer. Daily applications of 0.2% or 0.5% of DDVP provided excellent immediate control and kept animals entirely free of horn flies. Spraying on alternate days kept populations at a sub-annoying level. Similar results were obtained by spraying daily with 0.125-0.25% Ciodrin. Single applications of 0.5, 0.75, and 1.0% Ciodrin provided effective control for 10, 14, and 14 days, respectively.

In Mississippi, sprays of 0.5 or 1.0% malathion applied at the rate of one-half pint per head provided excellent control for 4 days and satisfactory control for about a week. Similar applications of 0.5% Cygon, methoxychlor, toxaphene and Famophos were effective for 3, 4-5, 4-5, and 5-6 days, respectively. Applications of 2 quarts per animal of 0.5% toxaphene or Co-ral provided effective control for 3-5 and 11 days, respectively. In tests with automatic sprayers, applications of 6 ounces (3 round trips per animal) in one day of 0.1% Baytex, 0.15% Delnav, 0.5% malathion, 0.5% toxaphene, 0.5% Bayer 22408, 1.0% Ciodrin, and 1.0% Ciodrin plus 0.25% DDVP provided effective control for 5-6 days. Ronnel and Baytex at 0.5% were effective 3-4 days. Similar applications of 0.1% Baytex, 0.5% Cygon and 0.5% Bayer 22408 were effective 1-2 days.

In Nebraska, 2 quart applications of 0.1 and 0.25% Stauffer R-1504 (ENT-25705) gave good control of horn flies for 11 days.

Tests were conducted to determine the effectiveness of low-level feeding of a number of materials in preventing horn fly breeding in cattle droppings. In Texas, daily feeding of Bayer 29493 at 1 mg./kg., Bayer 37341 at 2.5 mg./kg. and Bayer 37342 at 5 mg./kg. gave 100% control of larval breeding. Three commercial brands of Bacillus thuringensis administered at concentrations of 0.05-0.1% in feed also prevented fly breeding in droppings. These materials gave similar results in free choice feeding tests but the effective concentration of two of the materials was about 4 times that of the third one.

In field tests in Oregon, one-quart applications of 1% Shell SD-8436 (ENT-25840) gave highly effective control for over a week and 0.25% was moderately effective for this period. Similar applications of 0.25% Ciodrin, Bayer 29493, and Famophos, and 0.5% malathion, were effective for 4 days. Five insecticides were evaluated at high concentrations applied at low volumes (12 ml) to the backs of cattle with a pump oil can. In these tests 4% DDT was effective more than 21 days, 4% methoxyclor for 11-15 days, 4% malathion for 7-9 days, 4% Barthrin for 7 days and 5% Barthrin

for 14 days. In feeding tests, single oral administrations of 50 mg./kg. of ENT-25842 inhibited fly breeding in feces for 6-9 days. A similar dose of ENT-25840 was effective 4-5 days. A dose of only 1 mg./kg. of either compound was effective for 1 day. In tests involving feeding over a period of 5 days Zytron at 5 mg./kg. or carbophenothion at 10 mg./kg., prevented breeding in feces the first day. Lower doses of these materials and Bayer 29493 inhibited breeding after 3 days. Famophos 20 mg./kg. and Stauffer R1504 at 10 mg./kg. were not completely effective.

6. House Flies. Research was continued at Orlando, Fla., to develop more effective insecticides and other methods and materials for the control of house flies. Twenty-three new compounds were tested as space sprays in a wind tunnel against the regular susceptible colony and the Cradson (multiresistant) colony. Fourteen of the compounds were more effective against both the susceptible and resistant colonies than the standard, malathion. New insecticides were also evaluated as residual treatments against female house flies from the regular or Cradson colonies. The criterion of effectiveness was the number of weeks of aging during which the residues remained effective in killing house fly females. Against susceptible house flies Hercules AC-5727 alone and Bayer 39007 alone gave kills of 90% for one week, but in combination with Monsanto CP-16226, their effective periods were extended to 8 weeks and 12 weeks, respectively. The effectiveness of Hercules 7522H was also extended from 1 to 12 weeks with the addition of Monsanto CP-16226. Against flies of the Cradson colony all of these formulations failed before the fourth week. Against the regular colony Bayer 29952 and Bayer 30237 were effective for 64 and 56 weeks. Stauffer N-2230 and Stauffer N-2404 were 100% effective throughout 48 weeks of aging against the susceptible colony, but they were ineffective against the Cradson colony. General Chemical GC-3583 was still 100% effective after 96 weeks against the regular colony and Monsanto CP-40294 was effective for 24 weeks against the Cradson colony. Against house flies from the susceptible colony, General Chemical GC-4072 was 100% effective for 96 weeks as an acetone solution and Stauffer N-2310, Bayer 39197 and Monsanto CP-40273 for 48+ weeks. As wettable powder DDT was more than 90% effective for 48 weeks, Bayer 25141 for 48 weeks, Bayer 34098 for 40 weeks, Hooker HRS-1422 for 32 weeks, and Bayer 32651 for 28 weeks.

Residual tests (deposits of 100 mg./sq. ft.) were conducted with emulsions of diazinon, Baytex, and dimethoate against house flies in Florida dairy barns. The diazinon treatment failed to give satisfactory control as early as the first day after treatment. Baytex gave 97% control of 5 days and from 79 to 88% control through 14 days. Dimethoate gave controls ranging from 80 to 96% for 6 weeks, when the test was discontinued.

At Corvallis, Oreg., extensive studies were continued on the development of synergists that have been shown to overcome resistance to organophosphorus insecticides in both house flies and mosquitoes. Of some new types of compounds screened, results indicate that diisopropyl or dibutyl

substitutes would be most satisfactory. Selection of house flies with combinations of malathion and synergists are being carried out to determine if resistance to the combinations can be developed.

7. Cattle Grubs and Other Bots. Research was continued in Texas and Oregon to develop more effective insecticides for controlling cattle grubs and other bots affecting livestock. In Texas 79 new compounds were screened for systemic action by giving them orally (O), subcutaneously (SC) or intramuscularly (IM) at several dosages to guinea pigs infested with larvae of the screw-worm, Cochliomyia macellaria or Phormia regina. Nine of the materials showed systemic action in one or more types of administration. The active compounds and minimum effective dosages in mg./kg. were as follows: Stauffer R10094, 15 mg. O and 25 mg. SC; Shell SD-8448, 50 mg. SC; Shell SD 8530, 100 mg. O and 50 mg. SC; Chemagro S-8550, 100 mg. O; Hercules 7845, 10 mg. O and 25 mg. SC; Stauffer R-6032, 25 mg. O; Monsanto CP 19203, 10 mg. O and SC; Shell SD 8280, 10 mg. SC; and Pyramat, 100 mg.SC.

In Texas tests were conducted on small numbers of animals (2-4) with a number of compounds that had shown promise in screening tests or on individual cattle in 1961-1962 and with older effective materials administered in different ways. The effective materials, dosage, and route of administration were as follows: Co-ral, 2 and 8% pour-on; Bayer 37341, 2% pour-on; Bayer 37342, 2% pour-on, and 15 mg./kg. IM; Famophos, 20 mg./kg. IM; Stauffer R1504, 2% pour-on and 2.5 mg./kg. in feed for 10 days; Rhodia, 15 mg./kg. in feed for 10 days; Dipterex and Butonate, 5 mg./kg. in feed for 10 days; and Baytex, 2.5 mg./kg. in feed for 10 days. All of these treatments gave 97-100% control of the two species of grubs which infested the Wyoming cattle used in these tests.

Extensive field tests with Government and cooperator herds of cattle were conducted in Texas and Oregon to evaluate the effectiveness of promising new and several older systemics at different rates and various methods of administration. In Texas, a total of 30 materials were tested but only 7 produced 89-100% control. The effective materials, dosage and methods of application are as follows: Baytex, 1 mg./kg. in feed for 10 days; Dipterex, Butonate, and Bayer 37341, 2.5 mg. in feed for 10 days; Bayer 37342, 5 mg. in feed for 10 days; Famophos, 12.5% pour-on (65-125 mg. per animal); Imidan, 2% pour-on (125 mg.) and 10 mg./kg. IM.

In Oregon extensive tests were conducted with eight known systemics with the primary objective of determining the minimum effective dosage by pour-on and spraying. In pour-on tests, applications of 82 and 120 ml. of 12.5% Famophos in oil or glycol gave 99-100% control of grubs. Lesser amounts of these concentrations were slightly less effective (91-92% control). Oil applications of Famophos were slightly more effective than emulsions. Pour on applications of 60 and 120 ml. of 12.5% Bayer 37342 gave 95 and 96% control. With Bayer 29493 as little as 20 ml. of 10% in oil and 40 mg. of 5% in oil gave 92% control of grubs. Pour-on applications of 5 grams of Shell SD-8436 and 20 grams of Shell SD-8448 gave 100% control of grubs.

Oral administrations of 50 mg./kg. of both compounds failed to give satisfactory control.

In a series of spray tests, one gallon applications of 1, 1.5 and 2% Dipterex gave 97-100% control of grubs. Lower concentrations did not give satisfactory control. One-half % Ruelene sprays produced 93% control but at 0.25% control was only 80%. Sprays of Co-ral at 0.5% gave satisfactory control in only 1 of 3 tests. Bayer 29493 gave 100% control as a 0.25% spray but did not give satisfactory control at concentrations of 0.1 and 0.05%. A series of tests was conducted to compare the effectiveness of Famophos, Bayer 37342, Bayer 29493 and Ruelene at different dosages on a "per animal" basis rather than on body weight estimates. In these tests minimum dosages of Famophos at 10 grams per head, Bayer 37342 at 5 grams per head, Bayer 29439 at 2 grams per head and Ruelene at 2 grams per head provided 98-100% control of grubs in cattle of mixed sizes and ages. These results indicate that dosage applied on a per-animal basis is as satisfactory as dosages applied on body weight estimates.

8. Horse Flies and Deer Flies. In Mississippi tests were conducted to determine the effectiveness of synergized pyrethrum dusts containing 0.1 and 0.067% pyrethrins. Both formulations gave excellent protection from horse flies the day of treatment and fair protection for several days. Mist spray applications of 2 ounces of activated pyrethrum containing 0.62% pyrethrins were highly repellent to horse flies for 6 hours. Lower concentrations were proportionally less effective. Similar applications of 5% ENT-21195 provided complete protection for 6 hours and afforded 79% protection after 24 hours.

9. Lice. In field tests in Mississippi, eradication of cattle louse infestations was achieved with a single spraying of 0.5% General Chemical 4072 and 1% Sevin. Similar results against hog lice were obtained with sprays of 0.1 and 0.25% of GC 4072, 0.5 and 1.0% of Sevin, 0.25% of Ciodrin and 0.5% of Ciodrin, Dilan, and methoxychlor.

10. Ticks. Research on the control of ticks was confined to the Texas laboratory. Only 5 of 79 compounds screened for systemic effectiveness showed systemic action against lone star ticks engorging on treated guinea pigs. The effective materials, dosages (mg./kg.) and routes of administration were as follows: Monsanto CP-19203, 10 mg. O; Hercules 7845 and Stauffer R6032, 50 mg. O and SC; Shell 8280, 50 mg. SC; and Shell 8530, 100 mg. O.

In field tests, sprays of 0.5% toxaphene and 0.25% Stauffer 1504 gave excellent control of lone star ticks on cattle and were slightly better in both immediate and residual effectiveness than 0.025% diazinon, 0.05% carbophenothion, 0.1% Ciodrin and 0.25% Sevin and Dilan. In tests against the winter tick, complete control was obtained with sprays of 0.05% carbophenothion, 0.25% Bayer 37341, 0.1% Dipterex, 0.25% Dowco 175, and 0.3% Ciodrin. Diazinon at 0.05% gave excellent but incomplete control.

C. Insecticide Residue Determinations

1. Residue Studies. Studies were conducted in Texas on the absorption, distribution, storage and metabolic fate of insecticides in animals using chemical and radiometric methods of analysis. In Texas, steers were sprayed with 0.05 and 0.1% diazinon 1, 2, 6, and 10 times at weekly intervals and residue determinations made 6 days after the last spraying. One and two sprayings of 0.05% diazinon produced residues of only 0.05-0.09 p.p.m. in the fat. Six and 10 sprayings produced residues of 0.20-0.35 and 0.17-0.23, respectively. However, one day after the 10th spraying residue levels ranged from 0.53 to 0.85 p.p.m. Animals sprayed with 0.1% diazinon showed residues ranging from 0.47 to 0.83 p.p.m. There was very little difference in the residue levels created by 1, 2, 6, and 10 sprayings.

A Hereford calf was sprayed with C^{14}-labelled Stauffer R-1504 at a rate equivalent to 2 quarts of 0.5% Stauffer R-1504. The calf was slaughtered 7 days after spraying and samples of tissues were taken for analysis. Analyses were made by a radiometric method and by the chemical sulfide method. Residues found by the sulfide method were 0.04 p.p.m. in the omental fat, 0.11 p.p.m. in the subcutaneous fat, and less than 0.02 p.p.m. (sensitivity of method = $\pm$ 0.02 p.p.m.) in other tissues. The radiometric method (sensitivity of method $\pm$ 0.001 p.p.m.) gave values of 0.030 p.p.m. in the omental fat, 0.41 p.p.m. in the subcutaneous fat, 0.021 p.p.m. in renal fat, and 0.001 to 0.004 p.p.m. in the other tissues.

Residue determinations were made from 3 calves slaughtered 7, 16, and 28 days after spraying with 0.25% General Chemical 4072. The amounts of General Chemical 4072 found in the various tissues ranged from 0.004 to 0.0085 p.p.m. 7 days after spraying, 0.004 to 0.006 p.p.m. 16 days after spraying, and in all cases were 0.005 p.p.m. 28 days after spraying. A still unidentified material appeared in the liver of the calf slaughtered 7 days after treatment, and also in other tissues of the calves slaughtered 16 days and 28 days after treatment. It is possible that this material is a metabolite of General Chemical 4072.

2. Toxicity Studies. Work was conducted in Texas in cooperation with veterinarians of the Animal Disease and Parasite Research Division on the acute and chronic toxicity of insecticides and other materials. A summary of the results are presented. Detailed results will be given under Unit 2, Animal Diseases and Parasites (ADP a7-12, ADP a7-18, ADP a7-19, ADP a7-20, and ADP a7-23).

A colorimetric analytical method for studying the toxicology and presence in animal tissues of 2,4-D was successfully developed by using Carbon-14 labeled 2,4-D which could be determined with radiation detection instruments at each step of the developing technique. The analytical method which resulted is capable of detecting 0.05 parts per million of 2,4-D in animal tissue samples weighing 25 grams.

The ordinary feeding of Vitamin A to cattle increased their susceptibility to poisoning by Co-ral, particularly when they were also given phenothiazine drenches for internal parasite control. When impurities appeared in Co-ral, as they did during the fall of 1962, the toxicity was even greater. However, contaminated Co-ral, such as was credited with causing losses of cattle exceeding $750,000 in value, could not be shown to produce poisoning in Kerrville cattle unless Vitamin A and phenothiazine were also used. These initial studies need to be followed with others to determine whether the effect is limited to Co-ral or also follows the use of other insecticides.

More important than the increased incidence of poisoning when the Vitamin A and phenothiazine are present is the type of poisoning produced. Whereas Co-ral can poison and kill any animal, its activity normally is against an essential enzyme, cholinesterase. Poisoned animals that do not die rarely show any appreciable tissue changes. In the poisoning observed this year, there was a marked tissue change. One of the changes noted was a necrosis (death) of muscle fibers, particularly of the thigh muscles that compose the "round" meat cuts. In the living animal the necrosis is apparent as a lameness, in the carcass as an area of "white muscle" showing clearly against the normal red.

Four important enzyme systems were affected by the Vitamin A - phenothiazine - Co-ral combination. The significance of the effect is not yet clear.

Brahman cattle, and their crosses, were more susceptible to poisoning by Ciodrin and Compound 4072 than European breeds and their inter-crosses. The blood enzyme, cholinesterase, was more readily inhibited in Brahmans and their crosses than in European breeds. With Compound 4072, the only chemical given both by mouth and as a spray, the susceptibility was greater in Brahmans by both routes, indicating that the peculiarities of the Brahman skin were secondary to the species difference. The increased susceptibility was most marked to the compound Ciodrin.

In studies of the detoxication mechanisms in cattle and sheep, oximes, including 2-PAM chloride, DAM, and P_2S, were used to counteract poisoning by various organophosphorus compounds. The oximes are useful to cause a release of the enzyme (cholinesterase) inhibited by this group of pesticides. All three compounds were effective in mild or moderate poisoning. Their action was somewhat slow and atropine sulfate was still required in severe poisoning to gain time for the oximes to work.

Sodium selenite, sodium selenate, and d-alpha tocopheryl (Vitamin E) were effective in several instances of organophosphorus poisoning in our research. Studies are needed to explore the mechanisms by which these two substances exert their beneficial effects.

Studies of lindane sheep dips revealed that improper physical formulations were being employed, permitting the first sheep dipped in a fresh vat to

so deplete the fluid that the sheep dipped later were receiving extremely small concentrations in so far as control of parasites such as the scabies mites was concerned. The excessive amounts taken out by the first sheep dipped undoubtedly caused them to develop tissue residues far in excess of those normally to be expected.

Residues of 2,4-D in sheep fed the compound at a rate of 2 grams per head per day at Logan, Utah, were determined at Kerrville, Tex., using an analytical technique developed by the Kerrville staff. In sheep fed 30 daily doses, kidney, rumen, renal fat and body fat samples showed less apparent residue than did a control sheep. Muscle samples averaged less than 0.3 p.p.m. and liver samples less than 1.0 p.p.m. of 2,4-D.

In animals killed by the insect chemosterilants apholate, aphoxide, and methaphoxide, cytological changes were most prominent in the organs engaged in formation of the white cells of the blood. The changes indicated a severe decrease in ability of the parent tissue to supply the needs of the animals. In other studies, these materials were found to be highly cumulative in effect. Although some studies using low-level feeding are still current and the animals still alive, every dosage thus far tried has ultimately killed every sheep treated, in some cases after one year or more of exposure during which no observable illness occurred.

Thirty-two insecticides, most of them currently under test against livestock insects, were studied during the year. These studies furnished toxicological guidelines for decisions for further development of the materials. Co-ral and arsenic did not show potentiation in cattle and calves when used on the same day or within one or two days of one another. The study was conducted to show the safety of using both compounds on cattle imported from Mexico. Sprays of toxaphene following Co-ral sprayings seemed to reactivate the Co-ral deposits, leading to mild Co-ral poisoning in cattle and calves so treated.

Three fungicides and thirteen herbicides were studied in cattle. Generally, except for the mercurial fungicides, massive dosages repeated on several days were required to produce poisoning. Four herbicides, simazine, atrazine, bandane and promazine afflicted the nervous system. With bandane, a yearling steer died of a cerebral and medullary hemorrhage after showing various degrees of paralysis and other neurological symptomatology.

D. Biological Control

1. Mosquitoes. At Fresno, Calif., in cooperation with the Bureau of Vector Control, California State Board of Health, studies were initiated on the biological control of mosquitoes. Several species of microsporidia of the genus Thelohania killed mosquito larvae. Studies are under way to determine the host-parasite relationship.

2. Stable Fly and House Fly. Spalangia muscidarum, a pupal parasite that attacks stable flies, house flies and other Diptera, was reared in sufficient numbers in the laboratory at Lincoln, Nebr., to permit the start of systematic releases on May 16. An area of 16 square miles containing 20 farms was selected as a release area and 5 farms at another location were used for control observations. The release rate was varied from farm to farm according to the extent of the potential fly breeding areas. The release of this parasite, plus the natural occurrence of several other parasites, failed to produce marked reductions in stable fly or house fly populations. The percentage parasitism of pupae was erratic but at times very high. In general, planted pupae were more heavily parasitized than naturally occurring pupae. The percentage parasitism ranged from 0 to 100, without any apparent correlation with either time of year or the number of parasites released. In the course of these studies it was determined that three parasites parasitize stable fly pupae only, four parasitize both stable fly and house fly pupae, and four parasitize house fly pupae only.

3. Face Fly. In Nebraska observations were made on the effect of dung beetles on face fly larval breeding in cattle droppings in pastures. The constant movement of the beetles through fresh droppings reduced larval populations by about 80%.

E. Insect Sterility, Attractants and Other New Approaches to Control

1. Mosquitoes. In Oregon tepa and ENT-50450 were tested as sterilants against Culex tarsalis adults by spraying in wind tunnel tests and against larvae by exposure in water. Tepa sterilized males and females at a spray concentration of 6%; ENT-50450 sterilized only males at this concentration. Lower concentrations of either compound were not effective. Against larvae, both compounds were toxic at higher test concentrations and ineffective and partial toxicants at lower concentrations.

In Oregon studies have shown that grass infusion and log pond waters are attractive to ovipositing female Culex pipiens quinquefasciatus. Distilled water treated with methane or furfural was more attractive to ovipositing females than distilled water, but less attractive than log pond water.

2. Stable Fly. In Texas a small number of chemicals was evaluated by several methods as chemosterilants against the stable fly. Topical applications of 1.0 µg of ENT-26382 sterilized both sexes of flies. Similar applications of 4 µg of ENT-50569 sterilized males but did not completely sterilize the females. Three other materials--ENT-50042, 50396 and 50549--at 4 µg per fly, reduced oviposition and hatch but did not fully sterilize either sex.

In feeding tests, flies were completely sterilized when fed baits containing 0.2% aphoxide for 24 hours. Flies fed 0.1% aphoxide bait produced

normal numbers of eggs but only 2% hatched. Several materials were tested as residual treatments but none of them produced complete sterility.

Studies were conducted to determine the absorption, metabolism, and excretion of a P^{32}-labeled chemosterilant (MAPO) applied topically and in the diet of stable flies. When applied topically, the material was rapidly absorbed, with the females showing maximum absorption in 6 hours and the males in slightly longer time. The material was metabolized to the extent of about 60% in 24 hours. The main metabolic products were phosphoric acid and an unknown intermediate. Four other metabolites were isolated but not identified.

In the feeding tests MAPO was absorbed and distributed slower and in lesser amounts than in the topical tests. After 24 hours the degree of metabolism was about 50% higher than in the topical tests. In contrast to the topical tests, the main metabolite was an unknown intermediate which was about 4 times as abundant as phosphoric acid. Three of four other unknown intermediates occurred in greater amounts than phosphoric acid. Only about 8% of the applied dose of MAPO was excreted.

3. Face Fly. In Nebraska improved techniques were developed to screen materials as attractants for the face fly. Over 200 inorganic and organic materials were tested but none was as attractive as the liquid portion of fresh cattle manure.

A number of known chemosterilants was evaluated for effectiveness against the face fly. Flies consuming food containing 0.25% aphoxide for 24 hours were completely sterilized. Feeding for 3 days on a diet containing 0.0025% aphoxide prevented or greatly reduced oviposition and none of the eggs hatched. Males fed for 3 days on 0.005% aphoxide were incapable of fertilizing normal fertile females. Longevity of adults was not affected in these tests. ENT-26398, 50106 and 50107, at 1% in the diet produced complete sterility but were only partly effective at 0.25%.

4. Horn Fly. In Texas horn flies were sterilized by feeding overnight on a bait containing 0.05% tepa but a concentration of 0.01% was ineffective. Topical applications of 0.1 µg/fly fully sterilized both sexes of flies.

In Oregon adult horn flies were sterilized by exposing the pupae to 5000 r.

5. Screw-worm. In Texas where studies with the screw-worm were conducted, 57 of 350 compounds screened as candidate chemosterilants caused sterility when administered as topical treatments or fed to adults. Some of the compounds sterilized by both methods of administration. Secondary tests showed that some of these compounds sterilized one or both sexes completely, while others induced only partial sterilization or were ineffective. Tests with tretamine applied topically showed that adult flies could be sterilized when they were 1, 3, and 5 days old with equal facility. Males sterilized with thiotepa survived as well as untreated flies and competed

equally with untreated males in mating with females. However, the treated males were not as sexually competitive as untreated males. When a single dose of thiotepa or tretamine, adequate to sterilize either sex, was given in two half-doses 24 hours apart, survival of the flies was not improved and, further, a loss in sterilizing effectiveness occurred. Tretamine and thiotepa completely or partially sterilized screw-worms when puparia were immersed in solutions containing these compounds or were injected with them. With immersion, washing of puparia decreased the sterilizing effect indicating that adults obtained some or most of the sterilizing dose as they emerged from the puparium. Aerosol treatment of screw-worm adults with tretamine resulted in almost complete sterility.

Preliminary laboratory experiments indicated that ENT-50450 was as effective as gamma radiation in sterilizing screw-worm flies, and was superior in its lack of toxic side effects. Females mated to males sterilized with ENT-50450 continued to lay infertile eggs after the initial deposition of eggs indicating that sperm in spermathecae of females did not recover fertility. Males sterilized with ENT-50450 remained sterile throughout their lifetime.

Investigations with screw-worm flies and aziridinyl-type chemosterilants resulted in the conclusion that the primary influence of aziridinyl compounds on the ovaries of flies 0-4 hours old is the inhibition of oogensis, and of flies 1 day old, the induction of mutations. The effects of the aziridinyl compounds on the reproductive potential of female screw-worm flies were similar to those obtained with gamma radiation.

Studies were conducted to determine the absorption, metabolism and excretion of a P^{32}-labeled chemosterilant (MAPO) applied topically and in the diet of screw-worm flies. When applied topically the material was absorbed rather gradually. The absorbed material was metabolized to the extent of 42 and 58% in males and females, respectively, in 24 hours. The principal metabolic products were phosphoric acid and an unknown intermediate. Small amounts of five other intermediates were indicated but were not identified.

In the feeding tests, metepa was absorbed and distributed at about the same rates as in the topical tests. After 24 hours, the degree of metabolism in females was about the same as that in the topical test but, in the males, metabolism was 50% higher than in the topical tests. As in the feeding tests, the main metabolic products were phosphoric acid and an unknown intermediate. Five other intermediates were isolated but not identified. Approximately one-fifth of the applied dose of metepa was excreted.

Approximately 200 chemicals and other materials were screened as attractants for screw-worm flies. Of these, 10 were equal to or better than the standard liver bait and require further evaluation. Preliminary studies have indicated that mutant strains of screw-worm flies may respond

differently from normal strains to attractants since black-mutant flies were not attracted to the standard liver bait.

6. House Flies. Research on the development of sterilization for the control or eradication of house flies has been continued in Florida and Oregon. In Florida 886 new chemicals were screened for sterilant activity. Of these some produced toxic effects; however, 90 caused complete or partial sterility in the treated house flies. Seven compounds were highly effective in sterilizing both sexes of house flies, but two of these were also toxic at the dosages tested. Metepa and tepa as residual deposits on glass sterilized house flies at dosages from 25 to 250 mg. per sq. ft., but 5-fluoroorotic acid was ineffective as a residual deposit at similar dosages. Apholate in the adult food sterilized house flies regardless of their age and the flies did not regain fertility. Motile sperm were present in the testes of chemosterilized males and transferred during copulation to the spermathecae of females throughout the life span of the male.

Tests were initiated in Florida to determine whether a dosage of chemosterilant too low to prevent hatching or adult emergence might by the accumulation of small genetic injuries eventually reduce or eliminate reproduction. With apholate, one colony showed reduction of oviposition in the 4th, 5th, 6th, and 7th generations and no individuals reached the pupal stage in the 7th generation. With metepa, the 5th through 9th generation of another colony showed reduced oviposition and the 10th generation failed to oviposit.

Preliminary tests with apholate, tepa, and metepa, on house flies indicated that the probit of the percent sterility with house flies can be related to the log of the concentration.

Metepa and apholate shortened the life span of adult house flies considerably, but 90% or more of the male population survived the first 10 days, or that period of time in which mating activity is the greatest. Survival during the first 10 days was essentially the same in treated and non-treated house flies. Dipping house fly pupae in solutions of tepa, apholate, or metepa was toxic to most individuals, but a high degree of sterility occurred in individuals surviving the treatment.

Effective formulations of both dry baits and paint-on liquid baits have been developed.

A method has been devised for studying chromosomes of house flies. Using this technique, the effect of chemosterilants on spermatogensis and oogenesis will be made.

Studies conducted in Oregon with an olfactometer and with simulated treated fly models (pseudo flies) demonstrated the presence in female house flies of a volatile chemical or chemicals which can influence the

behavior of male flies. The behavior modification elicited was in the nature of attraction to a source of the pheromone, or an excitation of mating behavior patterns. The material which is benzene soluble and relatively stable was shown to be sex related, and appeared to be specific to the house fly since extracts of neither the face fly or stable fly affected the behavior of male house flies.

In Oregon tests were conducted to determine sterilizing effects of tepa and metepa on the little house fly (Fannia canicularis). All flies feeding on bait containing 0.1% tepa for 4 days and 0.5% tepa for 2 days succumbed in 3 and 5 days, respectively, and none oviposited before death. Flies feeding on 0.05% tepa for 3 days survived normally but did not oviposit. Metepa at 0.5% was highly toxic to flies. Flies feeding on baits containing 0.05 and 0.1% metepa survived and laid a few eggs but none hatched. Longevity of flies exposed for 2 hours on 100 mg. per sq. ft. residues of tepa was greatly reduced and all flies so treated were fully sterilized. Flies exposed on residues of 1 and 10 mg./sq. ft. laid a few eggs but none hatched. These tests indicate that the little house fly is much more easily sterilized than the house fly.

Over 50 inorganic and organic materials were tested as attractants for the little house fly. None showed significant attractancy.

In cooperation with the Agricultural Engineering and Animal Husbandry Research Divisions, studies were continued in new facilities at Beltsville, Md., to develop physical and mechanical methods of controlling house flies and other flies affecting cattle. Colonies of flies were established for conducting laboratory and outdoor cage studies. Studies to date have been concerned primarily with the attractiveness of various flies to different kinds and intensities of light. Black light ultraviolet radiation was attractive to both house flies and face flies during twilight periods. The use of fluorescent panels behind light sources increased their attractiveness.

7. Cattle Grubs. In Texas studies were made to determine the radiation dosage required to sterilize cattle grubs (Hypoderma lineatum). Complete sterilization was obtained by exposing pupae to 5000 r. A dosage of 2500 r sterilized the females but not the males.

8. Ticks. Extensive tests were conducted in Texas to determine the effects of different levels of radiation on different stages of the lone star tick. One series of tests with nymphs which had been engorged 2 weeks were exposed to different levels of radiation and the adults subsequently placed on hosts. Adults from nymphs exposed to 500 r or 1000 r engorged but produced no eggs. At a dose of 2500 r adults engorged normally and treated females mated with treated males did not oviposit. One of 8 treated females mated with normal males oviposited and some of the eggs hatched. When females and males treated with 5000 r were confined on hosts

neither sex engorged. Similar results were obtained with untreated males and treated females, but when treated males and untreated females were used, 2 of 3 females engorged and oviposited. One of the two egg masses obtained was not viable and the other showed only a partial hatch.

Exposure of newly emerged adult ticks to 1000 r and 2500 r did not affect engorgement and complete sterility was indicated in crosses of treated males and females and in crosses of untreated males and treated females. Untreated females mated with treated males produced eggs but none hatched. One of the ticks exposed to 5000 r engorged but it did not oviposit. No ticks engorged after treatment with dosages of 7500 r or 10,000 r.

Some of adult ticks dipped in concentrations of 0.25 and 0.5% of apholate, tepa, metepa, or tretamine were killed but those that survived engorged and laid viable eggs. Dipping in 1% concentrations of these materials also failed to produce complete sterility but the maximum viability of eggs from females treated with apholate was only 0.25%.

F. Evaluation of Equipment for Insect Detection and Control

1. In Texas, in cooperation with the Agricultural Engineering Research Division, studies were undertaken to develop control mechanisms for automatic spraying devices that would be stable and dependable and which would not excite cattle. Step-on electric switches (switch-mats) installed on the ground underneath the spray nozzles were superior to the usual electric eye switches. The step-on switches provided quick and reliable operation of sprayers and required only limited maintenance. Cattle were not aware of the imperceptible movement when they stepped on the mat and activated the sprayer. Most of the commercially-available automatic sprayers have mechanical defects and several adaptations were tried as improvements. One adaptation of the ring-type fitted with 7 nozzles proved superior to the commercial types.

At Beltsville, Md., in cooperation with the Agricultural Engineering and Animal Husbandry Research Divisions, work was initiated to develop test equipment and techniques of operation to evaluate the attractiveness of visible and ultraviolet radiation to house flies or other flies affecting cattle. This research has not yet reached a point of profitable summation.

G. Insect Vectors of Diseases

1. Anaplasmosis. Studies were continued in Texas, Mississippi, and Oregon to correlate the presence and abundance of insects and ticks with the incidence of anaplasmosis in herds of cattle. These studies were conducted in cooperation with the Animal Disease and Parasite Research Division and veterinarians of the various State experiment stations. In Texas, monthly surveys were continued to determine the abundance and identity of external parasites on infected (anaplasmosis) and clean herds

of cattle. Lone star ticks were abundant on cattle during May, June, and July, 1962, but declined rapidly in August and partially disappeared by September. During this same time, ear tick populations were high except for August. Horn flies became numerous early in May but periodical spraying of cattle minimized populations the remainder of the season. The winter tick and black-legged ticks appeared in November. The winter tick was abundant by December, but black-legged tick populations remained low. Moderate populations of the ear tick were present from October through December. Small numbers of lone star and black-legged ticks and large numbers of ear and winter ticks were present on cattle in January, 1963. In February and March, 1963, populations of the lone star tick increased, while the winter and black-legged ticks virtually disappeared. Ear tick populations remained high.

Prophylactic treatment with aureomycin minimized transmission of anaplasmosis and prevented acute cases. All of the 10 animals in the control herd contracted the disease and 3 of them had acute cases. At the low level (2 mg.) the maximum number of negative animals occurred after 3 months of treatment, whereas at the high level the maximum number occurred 3 months after treatment was terminated. In time, most of the negatives reverted to positives. The period of greatest transmission of the disease coincided with that in which the horse flies, Tabanus lineola and T. vittiger schwardti, and the mosquito, Psorophora confinnis, were most abundant.

In Maryland, hereditary transmission experiments and histopathological studies with the vectors of anaplasmosis of cattle were continued in cooperation with the Animal Disease and Parasite Research Division. Two series of transmission trials with the vector, Dermacentor andersoni, were conducted. In one, the F_1 larval and adult progeny of female ticks fed on an animal in the acute stages of anaplasmosis failed to transmit anaplasmosis to test calves. In tests with larval progeny, challenge of the animals with the disease proved the animals to be susceptible to the disease. Proof of the susceptibility of animals used with adult progeny has not been completed. In another series of trials, unmated and mated males fed on an animal with acute anaplasmosis were studied for survival under hibernating conditions (4-5°C. and 30-50% relative humidity with a photoperiod of 7 hours) and their ability to transmit anaplasmosis. Mated males survived only about 4 months in the hibernating environment and transmission trials could not be run. Eleven of 49 unmated males survived the 8-month-hibernating period, and 6 of these survived for transmission trials 3 months after the hibernating period. None of the survivors transmitted anaplasmosis to test cattle; however, not all of the animals have been proved susceptible by challenge at this time. In similar studies previously reported, transmission by infected males was demonstrated.

In Maryland, histopathological studies on Anaplasma-infected and non-infected Dermacentor andersoni were continued. Using immunofluorescence methods, anaplasmata were found in gut and excreta smear preparations from

ticks fed on infected animals. Brightfield and electron microscopic examinations revealed anaplasmata in excreta. Electron microscopic studies showed structures believed to be A. marginale in undigested erythrocytes. However, anaplasmata were not found in ultrathin section of the salivary glands and reproductive organs.

PUBLICATIONS REPORTING RESULTS OF USDA AND COOPERATIVE RESEARCH

Basic Biology, Physiology and Nutrition

Bigley, W. S. and Plapp, F. W. 1962. Metabolism of malathion and malaoxon by the mosquito Culex tarsalis Coq. Ins. Phys. 8: 545-57.
LaChance, L. E. and Leverich, A. P. 1962. Radiosensitivity of developing reproductive cells in female Callitroga hominivorax. Genetics 47: 721-35.
Harris, R. L. 1962. Laboratory colonization of the horn fly, Haematobia irritans (L.). Nature 196(4850): 191-2.
Eddy, G. W., Roth, A. R., and Plapp, F. W. 1962. Studies on the flight habits of some marked insects. Jour. Econ. Ent. 55: 603-7.
Lewis, L. F. and Eddy, G. W. 1962. The induced build-up of Bovicola bovis (L.) populations on cattle in Oregon. Jour. Econ. Ent. 55: 947-9.
Riley, J. and Hoffman, R. A. 1963. Observations on the meteorological-mosquito population relationship at Stoneville, Mississippi, 1959-60. Mosq. News 23: 36-40.
LaChance, L. E. and Bruns, S. B. 1963. Ovogenesis and radiosensitivity in Cochliomyia hominivorax (Diptera: Calliphoridae). Biol. Bull. 124: 65-83.

Insecticidal and Sanitation Control

Reed, L. B., Bushland, R. C., and Eddy, G. W. 1962. Systemic insecticides. 1962 Yearbook of Agriculture, pp: 340-3.
Roberts, R. H. 1962. Studies on the effect of radiation to pyrethrins applied to cattle. Jour. Econ. Ent. 55: 851-3.
Anthony, D. W. and Mott, L. O. 1963. Cattle lice eradication studies--a three-year evaluation. JAVMA 142: 130-2.
Drummond, R. O. 1963. Control of Gasterophilus larvae in horses with systemic insecticides. Jour. Econ. Ent. 56: 50-2.
Bushland, R. C. and Radeleff, R. D. 1963. Development of systemic insecticides for pests of animals. Ann. Rev. of Ent. 8: 215-38.
Chapman, H. C. and Lewallen, L. L. 1963. Field tests with insecticides against mountain Aedes larvae in California. Mosq. News 23: 41-4.
Jones, C. M. and Medley, J. G. 1963. Control of the face fly on cattle with Co-ral in grain and on pasture. Jour. Econ. Ent. 56: 214-5.
Hoffman, R. A. and Roberts, R. H. 1963. Horn fly control studies in Mississippi, 1961. Jour. Econ. Ent. 56: 258-61.

Insecticide Residue Determinations

Claborn, H. V., Roberts, R. H., Mann, H. D., Bowman, M. C., Ivey, M. C., Weidenbach, C. P., and Radeleff, R. D. 1963. Residues in body tissues of livestock sprayed with Sevin or given Sevin in the diet. Jour. Agr. and Food Chem. 11: 74-6.

Derbyshire, J. C., and Murphy, R. T. 1962. Diazinon residues in treated silage and milk of cows fed powdered diazinon. Jour. Agr. and Food Chem. 10: 384-6.

Jackson, J. B., Radeleff, R. D., Roberts, R. H., Hunt, L. M. and Buck, W. B. 1962. Acute toxicity of Delnav and its residues in tissues of livestock. Jour. Econ. Ent. 55: 699-702.

Radeleff, R. D., Jackson, J. B., Buck, W. G., Younger, R. L., Weidenbach, C. P., and Roberts, R. H. 1963. Toxicity studies of Bayer 21/199 in livestock. Jour. Amer. Vet. Med. Assoc. 142: 624-31.

Radeleff, R. D., Jackson, J. B., Hunt, L. M., Buck, W. B., and Wrich, M. W. 1963. The acute toxicity of ronnel applied dermally to cattle and sheep. Jour. Econ. Ent. 56: 272-4.

Insect Sterility, Attractants and Other New Approaches to Control

LaBrecque, G. C., Meifert, D. W., and Gouck, H. K. 1963. Effectiveness of three 2-methyl-aziridine derivatives as house fly chemosterilants. Fla. Ent. 46: 7-10.

LaBrecque, G. C., Meifert, D. W., and Fye, R. L. 1963. A field study on the control of house flies with chemosterilant techniques. Jour. Econ. Ent. 56: 150-2.

LaBrecque, G. C., Smith, C. N., and Meifert, D. W. 1962. A field experiment in the control of house flies with chemosterilant baits. Jour. Econ. Ent. 55: 449-50.

Weidhaas, D. E. 1962. Chemical sterilization of mosquitoes. Nature 195 (4843): 786-7.

LaChance, L. E., and Knipling, E. F. 1962. Control of insect populations through genetic manipulations. Ann. Ent. Soc. of America 55: 515-20.

Plapp, F. W., Jr., Bigley, W. S., Chapman, G. A., and Eddy, G. W. 1962. Metabolism of methaphoxide in mosquitoes, house flies, and mice. Jour. Econ. Ent. 55: 607-13.

Harris, R. L. 1962. Chemical induction of sterility in the stable fly. Jour. Econ. Ent. 55: 882-5.

LaChance, L. E. 1962. Effects of radiations on insects. Proc. North Cent. Br. ESA Meeting, XVII.

Graham, O. H. 1962. Research on chemosterilants for the control of livestock insects. Proc. North Cent. Br. ESA Meeting, XVII.

Insect Vectors of Diseases

Lindquist, A. W. 1962. Control of insect vectors of disease. Academic Press: 171-179.

Anthony, D. W. and Roby, T. O. 1962. Anaplasmosis transmission studies with Dermacentor variabilis (Say) and Dermacentor andersoni Stiles as experimental vectors. Proc. 4th Ann. Anaplasmosis Conf.: 78-81.

Hoffman, R. A. 1962. Control of vectors--equipment and insecticides. Proc. 4th Natl. Anaplasmosis Conf.: 84-5.

Roberts, R. H. 1962. Control of anaplasmosis through insect control, Washington County, Mississippi, 1961. Proc. 4th Natl. Anaplasmosis Conf.: 85-6.

AREA NO. 14. DAIRY CATTLE INSECTS

Problem. Flies, mosquitoes, grubs, lice, and ticks are common pests of dairy cattle that cause important losses in all parts of the United States. The screw-worm, a serious pest of dairy cattle, is prevalent in Texas, New Mexico, and several western States. Heavy attacks by biting flies lower milk production by 5 to 20%. Total losses to dairy cattle attributable to insects and ticks are estimated to exceed $200 million annually. Certain insect pests are also involved in the transmission of diseases of dairy cattle. Methods of control for dairy insects have received setbacks during recent years because the best available insecticides and most promising new materials produce residues in milk. In addition, house flies around dairy establishments have developed resistance to DDT and other insecticides. There is, therefore, great need to find safe, effective, nonresidue insecticides and repellents to control these insects and ticks. Effective systemic insecticides and ways of administration which would avoid residues are needed to combat grubs in dairy cattle and to prevent the face fly and horn fly from breeding in the manure. New approaches to control, including radiation and chemosterilants, need to be explored to determine their feasibility for the control of several dairy-cattle pests. Efforts also should be made to find and evaluate insect pathogens, parasites, and predators for controlling certain dairy-cattle pests. Expanded basic studies on the biology and physiology of these pests are needed to find weak links in their life cycle to serve as a basis for the development of more effective and safer methods of control. Research is also urgently needed on the role of insects in the spread of diseases of dairy cattle.

USDA PROGRAM

The Department has a continuing program involving basic and applied research on insects and ticks which affect the health and productivity of dairy cattle. Studies are conducted on the biology, physiology, genetics and nutrition of the screw-worm fly, stable fly, horn fly, horse and deer flies, the face fly, mosquitoes, and other pests; on the nature of insect resistance to insecticides; on the mode of action of insecticides and on their absorption, metabolism and excretion by insects; the effects of irradiation and chemosterilants on insects; insect attractants and repellents; and other new approaches to control. Research is directed towards the development of more effective conventional and systemic insecticides and protective treatments for the control of dairy-cattle pests. Studies are conducted to determine the occurrence of insecticide residues in the tissues and the milk of treated animals. Minor attention is given to the development of sanitation and management procedures and to biological control, especially parasites and predators, for controlling the face fly, stable fly, horse fly, and several other pests. Studies are conducted in cooperation with the Agricultural Engineering and Animal Husbandry Research Divisions to develop physical and mechanical methods of control, to evaluate traps and devices for estimating and

controlling natural insect populations and improved or special equipment for the application of insecticides to dairy cattle. Limited research is conducted on the role of insects and ticks as vectors of animal diseases, with special emphasis on bovine anaplasmosis. The research is conducted in major laboratories at Kerrville, Tex., Corvallis, Oreg., and Gainesville, Fla., (in June, 1963, investigations were transferred from Orlando, Fla., where the research herein reported was carried out) and at satellite stations at Beltsville, Md., Stoneville, Miss., Lincoln, Nebr., and Fresno, Calif.

The Federal scientific effort devoted to research in this area totals 15.8 professional man-years. Of this number 5.6 is devoted to basic biology, physiology and nutrition; 3.7 to insecticidal and sanitation control; 2.5 to insecticide residue determinations; 0.3 to biological control; 1.9 to insect sterility, attractants and other new approaches to control; 0.3 to evaluation of equipment for insect detection and control; 0.7 to insect vectors of diseases; and 0.8 to program leadership.

REPORT OF PROGRESS FOR USDA AND COOPERATIVE PROGRAMS

A. Basic Biology, Physiology and Nutrition

1. Mosquitoes. At Corvallis, Oreg., and Fresno, Calif., studies were continued on the biology and ecology of important mosquito species. In laboratory tests the majority of Culex tarsalis females preferred to feed at the higher of two feeding locations and preferred mice rather than chickens for their source of blood.

A study was conducted on the daily activity of several species of mosquitoes --Anopheles freeborni, Culex peus, Culiseta inornata, Culex incidens, and Culex tarsalis. All species behaved similarly. Both males and females began to move out of daytime resting shelters a few minutes before or after sunset. Only a few males and females (0.3 to 2.8%) remained in the shelters during the night. Adults did not begin to return to the shelters until sunrise or a few minutes later, and all did not return until sometime after 8:00 a.m. Exodus from and return to shelters appeared to be regulated by light intensity. Other factors such as preconditioning to light cycles, temperature, and humidity, may have had some effect since artificial lights did not prevent mosquitoes from leaving shelters eventually, but did change their time and rate of leaving. Use of special crosses of resistant and susceptible strains of Culex tarsalis showed that some of the females mate more than one time and utilize sperm from more than one mating.

Taxonomic studies defined suitable characters for separating dark-winged forms of Aedes dorsalis from A. melanimon and determined that these two forms coexist in only one area (Solano County, Calif.). Surveys at Borrego Springs (San Diego County, Calif.) were made to find isolated populations of mosquitoes for future studies on control through sterilization.

Surveys were continued on biting arthropods of the Humboldt River Basin in Nevada. During April, May, and June, water was plentiful and high populations of Aedes melanimon, A. dorsalis, A. vexans, Culex tarsalis, and Culiseta inornata were found. Mosquito larvae were found infected with a microsporidian, Thelohania sp. and a bacterium, both of which are under study as biological control agents. In late July, no floodwater Aedes breeding was observed, but moderate breeding of Culex tarsalis and Culiseta inornata was observed. Studies of mosquito larval populations in typical breeding areas in dairy drains and culverts in California during April indicated the presence of Culiseta inornata, Culiseta incidens, Culex tarsalis, Culex peus, Culex apicalis, and Anopheles franciscanus. No. breeding of Culex quinquefasciatus was found.

In Oregon, using C-14 labeled TDE, slight but consistent differences in the rate of degradation of TDE have been noted in susceptible and resistant strains of Culex tarsalis, indicating that the main source of resistance to this chemical is increased degradation of TDE to FW-152 and water soluble metabolites through an oxidative rather than a dehydrochlorination mechanism. Further tests with DDT and TDE have shown that analogs of DDT, which block metabolism via the oxidative pathway, overcome resistance to DDT and tend to confirm the hypothesis that resistance to DDT and TDE in tarsalis is due to a more rapid oxidative metabolism of the insecticide.

Early in July 1962, reports were received of a serious mosquito outbreak in the cattle-growing areas along the Gulf Coast of Louisiana and Texas. Surveys in these areas revealed a heavy mosquito population resulting from successive broods of mosquitoes over a period of 6-8 weeks. At the time of the survey, the spring calf crop had been decimated and hundreds of mature cattle had perished from the attacks of mosquitoes. Growers had sold or moved thousands of animals to avoid further losses. The loss incurred by cattlemen was estimated at $250,000. Because of the large area involved, control of the mosquitoes was not feasible.

2. Stable Fly. In Texas stable flies began to feed when they were only 4 hours old. The adults did not feed well or survive long on deer blood or several synthetic diets. They thrived on beef blood and beef serum but not on blood cells alone. At 80°F. and 50-60% relative humidity first matings occurred when the flies were 3 days old and 84% mated within 5 days. At 80°F. and 10-30% relative humidity mating did not begin until flies were 4 days old and only 12% mated in 5 days. No mating whatever occurred at 68°F. and uncontrolled humidity.

Stable fly larvae were not adversely affected by 17 hours of submergence in water, but 100% mortality resulted from 24 hours submergence. Exposure of pupae in water for 17 hours resulted in retarded development and greatly reduced adult emergence.

In Oregon field studies determined the dispersal pattern of marked adult stable flies. Flies were recovered over 14 miles from the point of release in 24-48 hours.

3. Face Fly. In Nebraska, face flies were first observed on cattle in the field on April 25 and by mid May they were present in annoying numbers. By early June heavy populations of 20 or more per head were widespread.

Studies on the behavior of the face fly were conducted in a large outdoor screened cage in which a calf was restrained. Several hundred laboratory-reared flies were released in the cage before each observation period. Less than 4% of the flies were present on the calf at any one time. The remainder rested in the grass or on the sides of the cage. Only an occasional male was observed on the calf. Nearly half of the flies congregated around the eyes and about 25% on the muzzle.

4. Horn Fly. In Texas, after considerable trial and error, a technique was developed for colonizing the horn fly in the laboratory without access to a bovine host. The diet consisted of 1 part bovine blood and 1 part beef juice plus 1 mg. of streptomycin, 1000 units of penicillin and 250 units of mycostatin per ml. of diet. Flies were fed by placing a cotton pad soaked with the diet on top of the holding cages. Cages consisted of 4" lengths of 6" plastic tubing with ends of plastic screen. Eggs were deposited on the plastic screen. Optimum conditions for adult reproduction were 90°F. temperature and 60-80% relative humidity. The colonization procedure is easy and inexpensive and greatly facilitates laboratory testing of insecticides, chemosterilants and repellents at all seasons of the year. Adult flies survived for 96 hours in a room kept at 75-80°F. and 62-67% relative humidity. Only a few survived in a room kept at 85-92°F. and 34-40% relative humidity.

In Oregon field studies determined the pattern of dispersal of marked adult horn flies. Flies were recovered over 7 miles from the point of release in 24-48 hours.

5. Screw-worm. Research on the screw-worm was discontinued at Kerrville, Tex., in September 1962 and moved to Mission, Tex., headquarters of the Southwest eradication campaign. This included research on the biology and ecology of the screw-worm under field conditions, vigor and longevity of sterilized flies, effects of radiation, the development of genetically marked strains, cytological studies with irradiated flies, nutrition, and special tests related to problems in the sterile male release program.

A new technique to determine the mating aggressiveness of sterilized or mutant screw-worm flies was developed. It was observed that in laboratory cages males harassed females sufficiently to cause mortality greater than that occurring when females were caged without males--in general, higher ratios of males to females caused greater mortality than lower ratios. Tests with various strains of screw-worms of known sexual competetiveness

in comparison with other mating aggressiveness tests confirmed the validity of the method. Since the criterion for mating aggressiveness is the mortality of females over time rather than egg production and viability, the time, space, and labor required to determine sexual aggressiveness were greatly reduced. Studies confirmed optimum numbers of screw-worm larvae per tray for rearing efficiency and optimum numbers of adults per cage for mating and longevity studies. They also confirmed diurnal periodicity in screw-worm pupae for emergence occurred from sunrise until noon. Studies indicated that the use of CO_2 as anesthesia for screw-worms is safe for handling young screw-worm adults for longevity and mating tests and scanning for genetic markers.

Research was continued to find and develop genetically distinct strains of screw-worm flies and to study these mutant flies. In the adults many genetic markers were found such as yellow eyes and white auxillary region ("Whaxy"). The genetic marker "Whaxy" affects the morphology of both the adult and larvae--a factor which would be invaluable in field studies. Approximately 270,000 screw-worm larvae were examined for morphological variants and from this 221 cultures were studied as possible genetic strains. Of markers found in the immature forms, at present three morphological characters--interrupted bands of segmental spines, two spiracular openings in the anal plates of third-instar larvae, and additional spines on the eleventh anterior segmental band--were demonstrated to be genetic in nature. None of these were established as a pure strain. Research with these and other mutant strains is being pursued to determine the genetics of the strains and if these strains are as vigorous and aggressive as the normal strain, factors which are essential if the strains are to be used in the release program. For example, preliminary genetic studies indicate that the "Whaxy" mutant is lethal in the homozygous state with only a few individuals escaping. Other strains have slight behavioral differences from the normal strain, i. e., they respond differently to attractants and survey traps.

When pupae of the screw-worm were irradiated in an atmosphere of CO_2 and air (50-50 mixture), damage to the reproductive system of the adult female was greater than that induced by a similar radiation treatment in air. When the pupae were pretreated in the gas mixture for approximately 45 minutes, complete sterility was induced by a treatment of 4,500 r delivered in CO_2 and air, whereas irradiation in air alone required about 5,500 to 6,200 r. Techniques were developed to study the cytological effects of radiation and chemosterilants on screw-worms, particularly on spermatogenesis and oogenesis. Preliminary studies revealed chromosomal aberrations severe enough to cause dominant lethality. Considerable data on oogenesis and spermatogenesis in normal screw-worm flies were accumulated as necessary background data for evaluating the effects of radiation and chemosterilants on reproduction.

For rearing larvae of the screw-worm, a synthetic diet was developed that contained casein, yeast extract, cholesterol, inorganic salts, water, and

agar. This medium was further defined by replacement of casein with a mixture of L-isomer amino acids, and of yeast extract with a mixture of B-vitamin and RNA. Larval growth and development on the defined medium were nearly equal to that on media containing casein and yeast. Larvae absolutely required thiamine, riboflavin, panthothenate, niacin, and choline for growth. Biotin and folic acid stimulated growth and were necessary for maturation to the adult stage. Pyridoxine and its analogs, pyridoxal and pyridoxamine, inositol, B_{12}, and carnitine had no effect on growth. Niacinamide spared niacin, but p- aminobenzoic acid had no effect on the folic acid requirement.

Studies on the effect of dessication, lack of adult food, reduced temperature, age of flies at time of release, and the effect of all of these factors on different strains of the screw-worm were undertaken to improve the survival of screw-worm flies released into the hot and dry climate of Texas. Provision of food in release cartons or the judicious use of reduced holding temperatures curtailed mortality prior to or shortly after release. Techniques need to be developed which would allow greater uniformity in age of flies at the time of release. Newly emerged flies carry a food and water reserve that will sustain them up to 24 hours (90°F. and 30% R.H.) compared to only 3 to 4 hours for 2-day-old flies. Selection of flies for individuals more capable of survival under unfavorable conditions has shown promising results.

Extensive studies were made of the ecology of screw-worm flies under field conditions by releasing tagged flies. It was determined that flies tend to congregate and disperse along water courses or streams and are capable of traveling long distances. This research resulted in the addition of stretegic releases of screw-worms along water courses and an increase in the effectiveness of the sterile-male release program.

6. House flies. At Orlando, Fla., research on control or eradication through the use of sterilization by radiation or chemicals has shown that considerable gaps exist in knowledge of the biology and mating behavior of house flies. Mating tests have shown that both males and females must undergo a sexual maturation time of at least 16 hours with males and a minimum of 24 hours with females. Once the mating drive has started, males will attempt to "strike" or mate with both males and females and certain inanimate objects, although they "strike" more readily and frequently with females. This fact along with experiments in a large cage-type chemotactometer suggested the presence of some type of a female sex attractant of a low order. Imperfect sex recognition in the male combined with a low order sex attractant in the female would account for the fact that males attempt to mate with either sex and result in the higher ratio of male-to-female "strikes." Quantitative data on the male and female mating behavior and a detailed description of the mating "strike" of the male and mating behavior of the male and female have been developed. Males or females which had their wings removed were able to mate with individuals of the opposite sex

with wings. Amputation of more than one pair of legs from the male prevented mating, while amputation of only one pair of legs impeded but did not prevent mating.

Tests were run to determine the actual time house flies remain in copula after initial seizure. Of 61 mating pairs that were trapped and observed, the shortest mating period was 44 minutes and longest, 96 minutes. The average was 60 minutes. Females examined after only 1 and 2 minutes of mating contained no sperm. Those examined after 3 to 5 minutes of mating had spermathecae which contained a few sperm and some which were completely filled. After mating for periods of 10 to 76 minutes, the spermathecae were filled to capacity with sperm, with only one exception.

Eighty-six female house flies reared in the laboratory laid an average of 94.4 eggs per female of which 89% hatched. The number of eggs laid varied from 12 to 186 per female. Under laboratory conditions some flies fed within an hour after emergence; others did not feed until later and some did not feed until they were 16 to 18 hours old.

At Corvallis, Oreg., studies were continued on the physiology of resistant and susceptible house flies. Isolan-resistant house flies have decreased ali-esterase activity and increased Isolan-detoxifying enzyme. Techniques using high-speed centrifugation have been developed which concentrate enzymes important in metabolizing or conferring resistance to organophosphorus compounds. In the case of malathion-resistant house flies, the nature of the alkyl groups attached directly to the phosphorus atom is the major factor in resistance. Thus, the primary cause of resistance is probably associated with the rate of recovery of the phosphorylated detoxifying enzyme after poisoning, rather than with an increased ability to cleave the toxic molecule per se.

In Oregon extensive studies were conducted on the biology, nutrition, and colonization of the little house fly (Fannia canicularis). After much trial and error a satisfactory rearing medium was developed which consisted of alfalfa meal, yeast extract solution and wood shavings. Adult flies were held in standard cages and provided with wrinkled balls of black muslin impregnated with alfalfa and yeast extracts for oviposition sites. Under such conditions 48% of the females and 25% of the males survived for 22 days in cages kept at 70-80°F. At 90°F. no males and only 8% of the females survived for 22 days. Egg deposition was about 5 times as great at 80°F. as at 70° or 90°F. Females began ovipositing in 5-6 days at 90°F., 8-9 days at 80°F., and 10-11 days at 70°F. The eggs dessicate rapidly and must be kept constantly on a moist surface to insure a high percentage of hatch.

7. Cattle Grubs. Research was continued in Texas and Oregon to determine the nutrition requirements of cattle grub larvae and to develop an artificial medium for rearing larvae under laboratory conditions. In Texas

various methods and media were tried in an effort to rear grub larvae. First stage larvae survived as long as 13-15 days in several tests but no second and third instars survived more than a week.

In Oregon, studies were conducted to determine the optimum conditions for larval pupation and development. Pupae held in moist sand at 40°, 44° and 50°F. produced no adults. Some emergence occurred at constant temperatures of 60° and 68°F. and at fluctuating temperatures ranging from 63°-75°F. Emergence was greater under fluctuating temperatures than at constant temperatures. Examination of pupae indicated that failure of adults to emerge was due to inability to eclose.

8. Horse Flies and Deer Flies. Studies were continued in Mississippi on the biology of tabanids. First adults of Hybomitra lasiophthalmus were noted on March 28. By mid-April this species was abundant and small numbers of T. vittiger schwardti and T. fusciacostatus were present. The usual succession of species appeared during the summer and early fall months but none reached their usual abundance. Population declined drastically during the latter part of July as a result of prolonged drouth. Populations rose only slightly following rains in late August.

9. Lice. Studies in Oregon showed that low louse populations on cattle during the summer months are largely due to destruction from licking. Short summer hair coats do not protect the lice, whereas long winter coats do. When short coated animals were restrained from licking themselves, heavy louse populations developed in 3-4 weeks.

10. Ticks. Observations in Texas showed that the lone star tick became active in January 1962, and population on cattle increased gradually during February, March and April, reaching a peak in May and June. Tick populations began to decline in July and had virtually disappeared by September.

The winter tick first appeared on cattle in October 1962, and populations gradually increased to a peak in December 1962, and January 1963. Tick populations declined rapidly in February and had virtually disappeared by late March.

B. Insecticidal and Sanitation Control

1. Mosquitoes. Studies were continued at Orlando, Fla., to find new and effective insecticides for the control of mosquitoes. In screening tests with Anopheles quadrimaculatus larvae, 28 of 81 compounds tested were rated Class IV in effectiveness. Four of these compounds--American Cyanamid CL-18133, Stauffer B-10046, Stauffer 8-10094, and Bayer 47940--were highly effective, killing 100% of the larvae at concentrations of 0.05 to 0.01 p.p.m. One hundred and nine plant extracts were also screened for toxicity, but none of these were toxic at low concentrations. Of 83 compounds tested against female Aedes taeniorhynchus in wind tunnel tests, 7 were as effective as the standard, malathion. Dimethrin in granular formulations was effective

in laboratory and field tests as a larvicide against Anopheles quadrimaculatus, Aedes aegypti, Aedes taeniorhynchus, and Culex quinquefasciatus mosquitoes. A mixture of DDT and an anti-resistant compound was no more effective than DDT alone against C. quinquefasciatus larvae.

In Oregon, studies were continued to find repellents for protecting animals from attacks by mosquitoes. Only one (ENT-26455) of 91 compounds tested was a highly effective repellent. This material showed 100% repellency for 24 hours and from 50 to 90% repellency after 48 hours. Several materials were highly effective toxicants but none was superior to the standard, Bayer 29493.

In Oregon, 46 compounds were tested for systemic action by giving them orally to mice and allowing mosquitoes to feed on the mice. At a dosage of 100 mg./kg., two compounds--Bayer 29493 and Shell SD 8436--killed all mosquitoes for 6 hours after the mice had been treated.

2. Stable Fly. In Texas, 240 compounds were screened in spot tests on cattle for repellency and toxicity against the stable fly. Of these materials, 6 were Class IV repellents at 5% and 21 were Class IV toxicants at 0.5%. A number of other materials were Class IV at higher concentrations. The outstanding repellents were ENT-20274, ENT-25927, ENT-26864, ENT-32965, ENT-25946, and ENT-27031.

Tests were conducted with the WHO test kit to determine the susceptibility of non-resistant stable flies to DDT and dieldrin. At an exposure of 30 minutes the LD-50s and LD-90s for DDT were 1.7 and 2.6% and for dieldrin 0.36 and 0.91%, respectively.

In field tests in Nebraska, applications of 1 pint of 0.5% Ciodrin to cattle gave good control of stable flies for 3-4 days. Daily applications with an automatic sprayer of 100 cc of 0.8% Ciodrin maintained very good control of stable flies. In field tests in Texas, sprays of 0.25% DOWCO 175 (ENT-25964) applied at the rate of 2 quarts per animal gave excellent control of stable flies for 3 days.

3. Face Fly. In Nebraska, tests were conducted in which materials were administered to cattle in feed in order to determine their effectiveness in preventing fly breeding in feces. Concentrations of 0.1% and higher of Bacillus thuringensis, an insect pathogen, prevented fly larval breeding in droppings. At concentrations of less than 0.1% larval survival increased in proportion to the decrease in concentration. Phenothiazine (2.5-3 microns) administered at the rate of 2 grams daily for 12 days gave erratic results but in no instance provided effective control of larval breeding. A concentration of 20 p.p.m. of Co-ral in feed completely inhibited larval breeding and DDVP at 0.5 mg./kg. was almost completely effective. Free choice consumption of salt containing 0.5 or 1.0% Co-ral gave erratic results, larval

control ranging from 40 to 85%. The erratic results were attributed to differences in consumption (0.06 to 0.35 mg./kg. daily) by individual animals.

In Nebraska, tests were conducted in which 4% malathion and 0.8% pyrethrins plus 1.1% piperonyl butoxide were installed around salt boxes. Daily contact with the dust bags caused reduction in face fly populations on cattle.

4. Horn Fly. Extensive field tests were conducted in Texas, Mississippi, Nebraska, and Oregon, to compare several old and promising new insecticides for the control of horn flies on cattle. In Texas sprays of 0.25 and 0.5% Hooker 1422 (ENT-25780) applied at the rate of 2 quarts per animal provided effective control of horn flies for 7-10 days. Applications of 2 quarts of 0.5% Ciodrin and 1 pint of 2% Ciodrin provided 14-95 days control in dry central Texas and 11-12 days control in humid east Texas. Tests were conducted with several materials applied in minimal amounts by an automatic mist sprayer. Daily applications of 0.2% or 0.5% of DDVP provided excellent immediate control and kept animals entirely free of horn flies. Spraying on alternate days kept populations at a sub-annoying level. Similar results were obtained by spraying daily with 0.125-0.25% Ciodrin. Single applications of 0.5, 0.75, and 1.0% Ciodrin provided effective control for 10, 14, and 14 days, respectively.

In Mississippi, sprays of 0.5 or 1.0% malathion applied at the rate of one-half pint per head provided excellent control for 4 days and satisfactory control for about a week. Similar applications of 0.5% Cygon, methoxychlor, toxaphene and Famophos were effective for 3, 4-5, 4-5, and 5-6 days, respectively. Applications of 2 quarts per animal of 0.5% toxaphene or Co-ral provided effective control for 3-5 and 11 days, respectively. In tests with automatic sprayers, applications of 6 ounces (3 round trips per animal) in one day of 0.1% Baytex, 0.15% Delnav, 0.5% malathion, 0.5% toxaphene, 0.5% Bayer 22408, 1.0% Ciodrin, and 1.0% Ciodrin plus 0.25% DDVP provided effective control for 5-6 days. Ronnel and Baytex at 0.5% were effective 3-4 days. Similar applications of 0.1% Baytex, 0.5% Cygon and 0.5% Bayer 22408 were effective 1-2 days.

In Nebraska, 2 quart applications of 0.1 and 0.25% Stauffer R-1504 (ENT-25705) gave good control of horn flies for 11 days.

Tests were conducted to determine the effectiveness of low-level feeding of a number of materials in preventing horn fly breeding in cattle droppings. In Texas, daily feeding of Bayer 29493 at 1 mg./kg., Bayer 37341 at 2.5 mg./kg. and Bayer 37342 at 5 mg./kg. gave 100% control of larval breeding. Three commercial brands of Bacillus thuringensis administered at concentrations of 0.05-0.1% in feed also prevented fly breeding in droppings. These materials gave similar results in free choice feeding tests but the effective concentration of two of the materials was about 4 times that of the third one.

In field tests in Oregon, one-quart applications of 1% Shell SD-8436 (ENT-25840) gave highly effective control for over a week and 0.25% was moderately effective for this period. Similar applications of 0.25% Ciodrin, Bayer 29493, and Famophos, and 0.5% malathion, were effective for 4 days. Five insecticides were evaluated at high concentrations applied at low volumes (12 ml) to the backs of cattle with a pump oil can. In these tests 4% DDT was effective more than 21 days, 4% methoxyclor for 11-15 days, 4% malathion for 7-9 days, 4% Barthrin from 7 days and 5% Barthrin for 14 days. In feeding tests, single oral administrations of 50 mg./kg. of ENT-25842 inhibited fly breeding in feces for 6-9 days. A similar dose of ENT-25840 was effective 4-5 days. A dose of only 1 mg./kg. of either compound was effective for 1 day. In tests involving feeding over a period of 5 days Zytron at 5 mg./kg. or carbophenothion at 10 mg./kg., prevented breeding in feces the first day. Lower doses of these materials and Bayer 29493 inhibited breeding after 3 days. Famophos 20 mg./kg. and Stauffer R1504 at 10 mg./kg. were not completely effective.

6. House Flies. Research was continued at Orlando, Fla., to develop more effective insecticides and other methods and materials for the control of house flies. Twenty-three new compounds were tested as space sprays in a wind tunnel against the regular susceptible colony and the Cradson (multiresistant) colony. Fourteen of the compounds were more effective against both the susceptible and resistant colonies than the standard, malathion. New insecticides were also evaluated as residual treatments against female house flies from the regular or Cradson colonies. The criterion of effectiveness was the number of weeks of aging during which the residues remained effective in killing house fly females. Against susceptible house flies Hercules AC-5727 alone and Bayer 39007 alone gave kills of 90% for one week, but in combination with Monsanto CP-16226, their effective periods were extended to 8 weeks and 12 weeks, respectively. The effectiveness of Hercules 7522H was also extended from 1 to 12 weeks with the addition of Monsanto CP-16226. Against flies of the Cradson colony all of these formulations failed before the fourth week. Against the regular colony Bayer 29952 and Bayer 30237 were effective for 64 and 56 weeks. Stauffer N-2230 and Stauffer N-2404 were 100% effective throughout 48 weeks of aging against the susceptible colony, but they were ineffective against the Cradson colony. General Chemical GC-3583 was still 100% effective after 96 weeks against the regular colony and Monsanto CP-40294 was effective for 24 weeks against the Cradson colony. Against house flies from the susceptible colony, General Chemical GC-4072 was 100% effective for 96 weeks as an acetone solution and Stauffer N-2310, Bayer 39197 and Monsanto CP-40273 for 48+ weeks. As wettable powder DDT was more than 90% effective for 48 weeks, Bayer 25141 for 48 weeks, Bayer 34098 for 40 weeks, Hooker HRS-1422 for 32 weeks, and Bayer 32651 for 28 weeks.

Residual tests (deposits of 100 mg./sq. ft.) were conducted with emulsions of diazinon, Baytex, and dimethoate against house flies in Florida dairy barns. The diazinon treatment failed to give satisfactory control as early

as the first day after treatment. Baytex gave 97% control of 5 days and from 79 to 88% control through 14 days. Dimethoate gave controls ranging from 80 to 96% for 6 weeks, when the test was discontinued.

At Corvallis, Oreg., extensive studies were continued on the development of synergists that have been shown to overcome resistance to organophosphorus insecticides in both house flies and mosquitoes. Of some new types of compounds screened, results indicate that diisopropyl or dibutyl substitutes would be most satisfactory. Selection of house flies with combinations of malathion and synergists are being carried out to determine if resistance to the combinations can be developed.

7. Cattle Grubs and Other Bots. Research was continued in Texas and Oregon to develop more effective insecticides for controlling cattle grubs and other bots affecting livestock. In Texas 79 new compounds were screened for systemic action by giving them orally (O), subcutaneously (SC) or intramuscularly (IM) at several dosages to guinea pigs infested with larvae of the screw-worm, Cochliomyia macellaria or Phormia regina. Nine of the materials showed systemic action in one or more types of administration. The active compounds and minimum effective dosages in mg./kg. were as follows: Stauffer R10094, 15 mg. O and 25 mg. SC; Shell SD-8448, 50 mg. SC; Shell SD 8530, 100 mg. O and 50 mg. SC; Chemagro S-8550, 100 mg. O; Hercules 7845, 10 mg. O and 25 mg. SC; Stauffer R-6032, 25 mg. O; Monsanto CP 19203, 10 mg. O and SC; Shell SD 8280, 10 mg. SC; and Pyramat, 100 mg. SC.

In Texas tests were conducted on small numbers of animals (2-4) with a number of compounds that had shown promise in screening tests or on individual cattle in 1961-1962 and with older effective materials administered in different ways. The effective materials, dosage, and route of administration were as follows: Co-ral, 2 and 8% pour-on; Bayer 37341, 2% pour-on; Bayer 37342, 2% pour-on, and 15 mg./kg. IM; Famophos, 20 mg./kg. IM; Stauffer R1504, 2% pour-on and 2.5 mg./kg. in feed for 10 days; Rhodia, 15 mg./kg. in feed for 10 days; Dipterex and Butonate, 5 mg./kg. in feed for 10 days; and Baytex, 2.5 mg./kg. in feed for 10 days. All of these treatments gave 97-100% control of the two species of grubs which infested the Wyoming cattle used in these tests.

Extensive field tests with Government and cooperator herds of cattle were conducted in Texas and Oregon to evaluate the effectiveness of promising new and several older systemics at different rates and various methods of administration. In Texas, a total of 30 materials were tested but only 7 produced 89-100% control. The effective materials, dosage and methods of application are as follows: Baytex, 1 mg./kg. in feed for 10 days; Dipterex, Butonate, and Bayer 37341, 2.5 mg. in feed for 10 days; Bayer 37342, 5 mg. in feed for 10 days; Famophos, 12.5% pour-on (65-125 mg. per animal); Imidan, 2% pour-on (125 mg.) and 10 mg./kg. IM.

In Oregon extensive tests were conducted with eight known systemics with the primary objective of determining the minimum effective dosage by pour-on and

spraying. In pour-on tests, applications of 82 and 120 ml. of 12.5% Famophos in oil or glycol gave 99-100% control of grubs. Lesser amounts of these concentrations were slightly less effective (91-92% control). Oil applications of Famophos were slightly more effective than emulsions. Pour-on applications of 60 and 120 ml. of 12.5% Bayer 37342 gave 95 and 96% control. With Bayer 29493 as little as 20 ml. of 10% in oil and 40 mg. of 5% in oil gave 92% control of grubs. Pour-on applications of 5 grams of Shell SD-8436 and 20 grams of Shell SD-8448 gave 100% control of grubs. Oral administrations of 50 mg./kg. of both compounds failed to give satisfactory control.

In a series of spray tests, one gallon applications of 1, 1.5 and 2% Dipterex gave 97-100% control of grubs. Lower concentrations did not give satisfactory control. One-half percent Ruelene sprays produced 93% control but at 0.25% control was only 80%. Sprays of Co-ral at 0.5% gave satisfactory control in only 1 of 3 tests. Bayer 29493 gave 100% control as a 0.25% spray but did not give satisfactory control at concentrations of 0.1 and 0.05%. A series of tests was conducted to compare the effectiveness of Famophos, Bayer 37342, Bayer 29493 and Ruelene at different dosages on a "per animal" basis rather than on body weight estimates. In these tests minimum dosages of Famophos at 10 grams per head, Bayer 37342 at 5 grams per head, Bayer 29439 at 2 grams per head and Ruelene at 2 grams per head provided 98-100% control of grubs in cattle of mixed sizes and ages. These results indicate that dosage applied on a per-animal basis is as satisfactory as dosages applied on body weight estimates.

8. Horse Flies and Deer Flies. In Mississippi tests were conducted to determine the effectiveness of synergized pyrethrum dusts containing 0.1 and 0.067% pyrethrins. Both formulations gave excellent protection from horse flies the day of treatment and fair protection for several days. Mist spray applications of 2 ounces of activated pyrethrum containing 0.62% pyrethrins were highly repellent to horse flies for 6 hours. Lower concentrations were proportionally less effective. Similar applications of 5% ENT-21195 provided complete protection for 6 hours and afforded 79% protection after 24 hours.

9. Lice. In field tests in Mississippi, eradication of cattle louse infestations was achieved with a single spraying of 0.5% General Chemical 4072 and 1% Sevin. Similar results against hog lice were obtained with sprays of 0.1 and 0.25% of GC 4072, 0.5 and 1.0% of Sevin, 0.25% of Ciodrin and 0.5% of Ciodrin, Dilan, and methoxychlor.

10. Ticks. Research on the control of ticks was confined to the Texas laboratory. Only 5 of 79 compounds screened for systemic effectiveness showed systemic action against lone star ticks engorging on treated guinea pigs. The effective materials, dosages (mg./kg.) and routes of administration were as follows: Monsanto CP-19203, 10 mg. O; Hercules 7845 and Stauffer R6032, 50 mg. O and SC; Shell 8280, 50 mg. SC; and Shell 8530, 100 mg. O.

In field tests, sprays of 0.5% toxaphene and 0.25% Stauffer 1504 gave excellent control of lone star ticks on cattle and were slightly better in both immediate and residual effectiveness than 0.025% diazinon, 0.05% carbophenothion, 0.1% Ciodrin and 0.25% Sevin and Dilan. In tests against the winter tick, complete control was obtained with sprays of 0.05% carbophenothion, 0.25% Bayer 37341, 0.1% Dipterex, 0.25% Dowco 175, and 0.3% Ciodrin. Diazinon at 0.05% gave excellent but incomplete control.

C. Insecticide Residue Determinations

1. Residue Studies. Studies were conducted in Texas on the absorption, distribution, storage and metabolic fate of insecticides in animals using chemical and radiometric methods of analysis. In Texas, steers were sprayed with 0.05 and 0.1% diazinon 1, 2, 6, and 10 times at weekly intervals and residue determinations made 6 days after the last spraying. One and two sprayings of 0.05% diazinon produced residues of only 0.05-0.09 p.p.m. in the fat. Six and 10 sprayings produced residues of 0.20-0.35 and 0.17-0.23, respectively. However, one day after the 10th spraying residue levels ranged from 0.53 to 0.85 p.p.m. Animals sprayed with 0.1% diazinon showed residues ranging from 0.47 to 0.83 p.p.m. There was very little difference in the residue levels created by 1, 2, 6, and 10 sprayings.

A Hereford calf was sprayed with C^{14}-labeled Stauffer R-1504 at a rate equivalent to 2 quarts of 0.5% Stauffer R-1504. The calf was slaughtered 7 days after spraying and samples of tissues were taken for analysis. Analyses were made by a radiometric method and by the chemical sulfide method. Residues found by the sulfide method were 0.04 p.p.m. in the omental fat, 0.11 p.p.m. in the subcutaneous fat, and less than 0.02 p.p.m. (sensitivity of method = $\pm$ 0.02 p.p.m.) in other tissues. The radiometric method (sensitivity of method $\pm$ 0.001 p.p.m.) gave values of 0.030 p.p.m. in the omental fat, 0.41 p.p.m. in the subcutaneous fat, 0.021 p.p.m. in renal fat, and 0.001 to 0.004 p.p.m. in the other tissues.

Residue determinations were made from 3 calves slaughtered 7, 16, and 28 days after spraying with 0.25% General Chemical 4072. The amounts of General Chemical 4072 found in the various tissues ranged from 0.004 to 0.0085 p.p.m. 7 days after spraying, 0.004 to 0.006 p.p.m. 16 days after spraying, and in all cases were 0.005 p.p.m. 28 days after spraying. A still unidentified material appeared in the liver of the calf slaughtered 7 days after treatment, and also in other tissues of the calves slaughtered 16 days and 28 days after treatment. It is possible that this material is a metabolite of General Chemical 4072.

2. Toxicity Studies. Work was conducted in Texas in cooperation with veterinarians of the Animal Disease and Parasite Research Division on the acute and chronic toxicity of insecticides and other materials. A summary of the results are presented. Detailed results will be given under Unit 2, Animal Diseases and Parasites (ADP a7-12, ADP a7-18, ADP a7-19, ADP a7-20, and ADP a7-23).

A colorimetric analytical method for studying the toxicology and presence in animal tissues of 2,4-D was successfully developed by using Carbon-14 labeled 2,4-D which could be determined with radiation detection instruments at each step of the developing technique. The analytical method which resulted is capable of detecting 0.05 parts per million of 2,4-D in animal tissue samples weighing 25 grams.

The ordinary feeding of Vitamin A to cattle increased their susceptibility to poisoning by Co-ral, particularly when they were also given phenothiazine drenches for internal parasite control. When impurities appeared in Co-ral, as they did during the fall of 1962, the toxicity was even greater. However, contaminated Co-ral, such as was credited with causing losses of cattle exceeding $750,000 in value, could not be shown to produce poisoning in Kerrville cattle unless Vitamin A and phenothiazine were also used. These initial studies need to be followed with others to determine whether the effect is limited to Co-ral or also follows the use of other insecticides.

More important than the increased incidence of poisoning when the Vitamin A and phenothiazine are present is the type of poisoning produced. Whereas Co-ral can poison and kill any animal, its activity normally is against an essential enzyme, cholinesterase. Poisoned animals that do not die rarely show any appreciable tissue changes. In the poisoning observed this year, there was a marked tissue change. One of the changes noted was a necrosis (death) of muscle fibers, particularly of the thigh muscles that compose the "round" meat cuts. In the living animal the necrosis is apparent as a lameness, in the carcass as an area of "white muscle" showing clearly against the normal red.

Four important enzyme systems were affected by the Vitamin A - phenothiazine - Co-ral combination. The significance of the effect is not yet clear.

Brahman cattle, and their crosses, were more susceptible to poisoning by Ciodrin and Compound 4072 than European breeds and their inter-crosses. The blood enzyme, cholinesterase, was more readily inhibited in Brahmans and their crosses than in European breeds. With Compound 4072, the only chemical given both by mouth and as a spray, the susceptibility was greater in Brahmans by both routes, indicating that the peculiarities of the Brahman skin were secondary to the species difference. The increased susceptibility was most marked to the compound Ciodrin.

In studies of the detoxication mechanisms in cattle and sheep, oximes, including 2-PAM chloride, DAM, and P_2S, were used to counteract poisoning by various organophosphorus compounds. The oximes are useful to cause a release of the enzyme (cholinesterase) inhibited by this group of pesticides. All three compounds were effective in mild or moderate poisoning. Their action was somewhat slow and atropine sulfate was still required in severe poisoning to gain time for the oximes to work.

Sodium selenite, sodium selenate, and d-alpha tocopheryl (Vitamin E) were effective in several instances of organophosphorus poisoning in our research. Studies are needed to explore the mechanisms by which these two substances exert their beneficial effects.

Thirty-two insecticides, most of them currently under test against livestock insects, were studied during the year. These studies furnished toxicological guidelines for decisions for further development of the materials. Co-ral and arsenic did not show potentiation in cattle and calves when used on the same day or within one or two days of one another. The study was conducted to show the safety of using both compounds on cattle imported from Mexico. Sprays of toxaphene following Co-ral sprayings seemed to reactivate the Co-ral deposits, leading to mild Co-ral poisoning in cattle and calves so treated.

Three fungicides and thirteen herbicides were studied in cattle. Generally, except for the mercurial fungicides, massive dosages repeated on several days were required to produce poisoning. Four herbicides, simazine, atrazine, bandane and promazine afflicted the nervous system. With bandane, a yearling steer died of a cerebral and medullary hemorrhage after showing various degrees of paralysis and other neurological symptomatology.

D. Biological Control

1. Mosquitoes. At Fresno, Calif., in cooperation with the Bureau of Vector Control, California State Board of Health, studies were initiated on the biological control of mosquitoes. Several species of microsporidia of the genus Thelohania killed mosquito larvae. Studies are under way to determine the host-parasite relationship.

2. Stable Fly and House Fly. Spalangia muscidarum, a pupal parasite that attacks stable flies, house flies and other Diptera, was reared in sufficient numbers in the laboratory at Lincoln, Nebr., to permit the start of systematic releases on May 16. An area of 16 square miles containing 20 farms was selected as a release area and 5 farms at another location were used for control observations. The release rate was varied from farm to farm according to the extent of the potential fly breeding areas. The release of this parasite, plus the natural occurrence of several other parasites, failed to produce marked reductions in stable fly or house fly populations. The percentage parasitism of pupae was erratic but at times very high. In general, planted pupae were more heavily parasitized than naturally occurring pupae. The percentage parasitism ranged from 0 to 100, without any apparent correlation with either time of year or the number of parasites released. In the course of these studies it was determined that three parasites parasitize stable fly pupae only, four parasitize both stable fly and house fly pupae, and four parasitize house fly pupae only.

3. Face Fly. In Nebraska observations were made on the effect of dung beetles on face fly larval breeding in cattle droppings in pastures. The constant movement of the beetles through fresh droppings reduced larval populations by about 80%.

E. Insect Sterility, Attractants and Other New Approaches to Control

1. Mosquitoes. In Oregon tepa and ENT-50450 were tested as sterilants against Culex tarsalis adults by spraying in wind tunnel tests and against larvae by exposure in water. Tepa sterilized males and females at a spray concentration of 6%; ENT-50450 sterilized only males at this concentration. Lower concentrations of either compound were not effective. Against larvae, both compounds were toxic at higher test concentrations and ineffective and partial toxicants at lower concentrations.

In Oregon studies have shown that grass infusion and log pond waters are attractive to ovipositing female Culex pipiens quinquefasciatus. Distilled water treated with methane or furfural was more attractive to ovipositing females than distilled water, but less attractive than log pond water.

2. Stable Fly. In Texas a small number of chemicals was evaluated by several methods as chemosterilants against the stable fly. Topical applications of 1.0 µg of ENT-26382 sterilized both sexes of flies. Similar applications of 4 µg of ENT-50569 sterilized males but did not completely sterilize the females. Three other materials--ENT-50042, 50396 and 50549--at 4 µg per fly, reduced oviposition and hatch but did not fully sterilize either sex.

In feeding tests, flies were completely sterilized when fed baits containing 0.2% aphoxide for 24 hours. Flies fed 0.1% aphoxide bait produced normal numbers of eggs but only 2% hatched. Several materials were tested as residual treatments but none of them produced complete sterility.

Studies were conducted to determine the absorption, metabolism and excretion of a P^{32}-labeled chemosterilant (MAPO) applied topically and in the diet of stable flies. When applied topically, the material was rapidly absorbed, with the females showing maximum absorption in 6 hours and the males in slightly longer time. The material was metabolized to the extent of about 60% in 24 hours. The main metabolic products were phosphoric acid and an unknown intermediate. Four other metabolites were isolated but not identified.

In the feeding tests MAPO was absorbed and distributed slower and in lesser amounts than in the topical tests. After 24 hours the degree of metabolism was about 50% higher than in the topical tests. In contrast to the topical tests, the main metabolite was an unknown intermediate which was about 4 times as abundant as phosphoric acid. Three of four other unknown intermediates occurred in greater amounts than phosphoric acid. Only about 8% of the applied dose of MAPO was excreted.

3. Face Fly. In Nebraska improved techniques were developed to screen materials as attractants for the face fly. Over 200 inorganic and organic materials were tested but none was as attractive as the liquid portion of fresh cattle manure.

A number of known chemosterilants was evaluated for effectiveness against the face fly. Flies consuming food containing 0.25% aphoxide for 24 hours were completely sterilized. Feeding for 3 days on a diet containing 0.0025% aphoxide prevented or greatly reduced oviposition and none of the eggs hatched. Males fed for 3 days on 0.005% aphoxide were incapable of fertilizing normal fertile females. Longevity of adults was not affected in these tests. ENT-26398, 50106 and 50107, at 1% in the diet produced complete sterility but were only partly effective at 0.25%.

4. Horn Fly. In Texas horn flies were sterilized by feeding overnight on a bait containing 0.05% tepa but a concentration of 0.01% was ineffective. Topical applications of 0.1 µg/fly fully sterilized both sexes of flies.

In Oregon adult horn flies were sterilized by exposing the pupae to 5000 r.

5. Screw-worm. In Texas where studies with the screw-worm were conducted, 57 of 350 compounds screened as candidate chemosterilants caused sterility when administered as topical treatments or fed to adults. Some of the compounds sterilized by both methods of administration. Secondary tests showed that some of these compounds sterilized one or both sexes completely, while others induced only partial sterilization or were ineffective. Tests with tretamine applied topically showed that adult flies could be sterilized when they were 1, 3, and 5 days old with equal facility. Males sterilized with thiotepa survived as well as untreated flies and competed equally with untreated males in mating with females. However, the treated males were not as sexually competitive as untreated males. When a single dose of thiotepa or tretamine, adequate to sterilize either sex, was given in two half-doses 24 hours apart, survival of the flies was not improved and, further, a loss in sterilizing effectiveness occurred. Tretamine and thiotepa completely or partially sterilized screw-worms when puparia were immersed in solutions containing these compounds or were injected with them. With immersion, washing of puparia decreased the sterilizing effect, indicating that adults obtained some or most of the sterilizing dose as they emerged from the puparium. Aerosol treatment of screw-worm adults with tretamine resulted in almost complete sterility.

Preliminary laboratory experiments indicated that ENT-50450 was as effective as gamma radiation in sterilizing screw-worm flies, and was superior in its lack of toxic side effects. Females mated to males sterilized with ENT-50450 continued to lay infertile eggs after the initial deposition of eggs indicating that sperm in spermathecae of females did not recover fertility. Males sterilized with ENT-50450 remained sterile throughout their lifetime.

Investigations with screw-worm flies and aziridinyl-type chemosterilants resulted in the conclusion that the primary influence of aziridinyl compounds on the ovaries of flies 0-4 hours old is the inhibition of oogensis, and of flies 1 day old, the induction of mutuations. The effects of the aziridinyl compounds on the reproductive potential of female screw-worm flies were similar to those obtained with gamma radiation.

Studies were conducted to determine the absorption, metabolism and excretion of a P^{32}-labeled chemosterilant (MAPO) applied topically and in the diet of screw-worm flies. When applied topically the material was absorbed rather gradually. The absorbed material was metabolized to the extent of 42 and 58% in males and females, respectively, in 24 hours. The principal metabolic products were phosphoric acid and an unknown intermediate. Small amounts of five other intermediates were indicated but were not identified.

In the feeding tests, metepa was absorbed and distributed at about the same rates as in the topical tests. After 24 hours, the degree of metabolism in females was about the same as that in the topical test but, in the males, metabolism was 50% higher than in the topical tests. As in the feeding tests, the main metabolic products were phosphoric acid and an unknown intermediate. Five other intermediates were isolated but not identified. Approximately one-fifth of the applied dose of metepa was excreted.

Approximately 200 chemicals and other materials were screened as attractants for screw-worm flies. Of these, 10 were equal to or better than the standard liver bait and require further evaluation. Preliminary studies have indicated that mutant strains of screw-worm flies may respond differently from normal strains to attractants since black-mutant flies were not attracted to the standard liver bait.

6. House Flies. Research on the development of sterilization for the control or eradication of house flies has been continued in Florida and Oregon. In Florida 886 new chemicals were screened for sterilant activity. Of these some produced toxic effects; however, 90 caused complete or partial sterility in the treated house flies. Seven compounds were highly effective in sterilizing both sexes of house flies, but two of these were also toxic at the dosages tested. Metepa and tepa as residual deposits on glass sterilized house flies at dosages from 25 to 250 mg. per sq. ft., but 5-fluoroorotic acid was ineffective as a residual deposit at similar dosages. Apholate in the adult food sterilized house flies regardless of their age and the flies did not regain fertility. Motile sperm were present in the testes of chemosterilized males and transferred during copulation to the spermathecae of females throughout the life span of the male.

Tests were initiated in Florida to determine whether a dosage of chemosterilant too low to prevent hatching or adult emergence might by the accumulation of small genetic injuries eventually reduce or eliminate reproduction. With apholate, one colony showed reduction of oviposition in the 4th, 5th, 6th, and 7th generations and no individuals reached the pupal stage in the 7th

generation. With metepa, the 5th through 9th generation of another colony showed reduced oviposition and the 10th generation failed to oviposit.

Preliminary tests with apholate, tepa, and metepa, on house flies indicated that the probit of the percent sterility with house flies can be related to the log of the concentration.

Metepa and apholate shortened the life span of adult house flies considerably, but 90% or more of the male population survived the first 10 days, or that period of time in which mating activity is the greatest. Survival during the first 10 days was essentially the same in treated and non-treated house flies. Dipping house fly pupae in solutions of tepa, apholate, or metepa was toxic to most individuals, but a high degree of sterility occurred in individuals surviving the treatment.

Effective formulations of both dry baits and paint-on liquid baits have been developed.

A method has been devised for studying chromosomes of house flies. Using this technique, the effect of chemosterilants on spermatogensis and oogensis will be made.

Studies conducted in Oregon with an olfactometer and with simulated treated fly models (pseudo flies) demonstrated the presence in female house flies of a volatile chemical or chemicals which can influence the behavior of male flies. The behavior modification elicited was in the nature of attraction to a source of the pheromone, or an excitation of mating behavior patterns. The material which is benzene soluble and relatively stable was shown to be sex related, and appeared to be specific to the house fly since extracts of neither the face fly or stable fly affected the behavior of male house flies.

In Oregon tests were conducted to determine sterilizing effects of tepa and metepa on the little house fly (Fannia canicularis). All flies feeding on bait containing 0.1% tepa for 4 days and 0.5% tepa for 2 days succumbed in 3 and 5 days, respectively, and none oviposited before death. Flies feeding on 0.05% tepa for 3 days survived normally but did not oviposit. Metepa at 0.5% was highly toxic to flies. Flies feeding on baits containing 0.05 and 0.1% metepa survived and laid a few eggs but none hatched. Longevity of flies exposed for 2 hours on 100 mg. per sq. ft. residues of tepa was greatly reduced and all flies so treated were fully sterilized. Flies exposed on residues of 1 and 10 mg./sq. ft. laid a few eggs but none hatched. These tests indicate that the little house fly is much more easily sterilized than the house fly.

Over 50 inorganic and organic materials were tested as attractants for the little house fly. None showed significant attractancy.

In cooperation with the Agricultural Engineering and Animal Husbandry Reseach Divisions, studies were continued in new facilities at Beltsville, Md., to

develop physical and mechanical methods of controlling house flies and other flies affecting cattle. Colonies of flies were established for conducting laboratory and outdoor cage studies. Studies to date have been concerned primarily with the attractiveness of various flies to different kinds and intensities of light. Black light ultraviolet radiation was attractive to both house flies and face flies during twilight periods. The use of fluorescent panels behind light sources increased their attractiveness.

7. Cattle Grubs. In Texas studies were made to determine the radiation dosage required to sterilize cattle grubs (Hypoderma lineatum). Complete sterilization was obtained by exposing pupae to 5000 r. A dosage of 2500 r sterilized the females but not the males.

8. Ticks. Extensive tests were conducted in Texas to determine the effects of different levels of radiation on different stages of the lone star tick. One series of tests with nymphs which had been engorged 2 weeks were exposed to different levels of radiation and the adults subsequently placed on hosts. Adults from nymphs exposed to 500 r or 1000 r engorged but produced no eggs. At a dose of 2500 r adults engorged normally and treated females mated with treated males did not oviposit. One of 8 treated females mated with normal males oviposited and some of the eggs hatched. When females and males treated with 5000 r were confined on hosts neither sex engorged. Similar results were obtained with untreated males and treated females, but when treated males and untreated females were used, 2 of 3 females engorged and oviposited. One of the two egg masses obtained was not viable and the other showed only a partial hatch.

Exposure of newly emerged adult ticks to 1000 r and 2500 r did not affect engorgement and complete sterility was indicated in crosses of treated males and females and in crosses of untreated males and treated females. Untreated females mated with treated males produced eggs but none hatched. One of the ticks exposed to 5000 r engorged but it did not oviposit. No ticks engorged after treatment with dosages of 7500 r or 10,000 r.

Some of adult ticks dipped in concentrations of 0.25 and 0.5% of apholate, tepa, metepa, or tretamine were killed but those that survived engorged and laid viable eggs. Dipping in 1% concentrations of these materials also failed to produce complete sterility but the maximum viability of eggs from females treated with apholate was only 0.25%.

F. Evaluation of Equipment for Insect Detection and Control

1. In Texas, in cooperation with the Agricultural Engineering Research Division, studies were undertaken to develop control mechanisms for automatic spraying devices that would be stable and dependable and which would not excite cattle. Step-on electric switches (switch-mats) installed on the ground underneath the spray nozzles were superior to the usual electric eye switches. The step-on switches provided quick and reliable operation of sprayers and required only limited maintenance. Cattle were not aware of

the imperceptible movement when they stepped on the mat and activated the sprayer. Most of the commercially-available automatic sprayers have mechanical defects and several adaptations were tried as improvements. One adaptation of the ring-type fitted with 7 nozzles proved superior to the commercial types.

At Beltsville, Md., in cooperation with the Agricultural Engineering and Animal Husbandry Research Division, work was initiated to develop test equipment and techniques of operation to evaluate the attractiveness of visible and ultraviolet radiation to house flies or other flies affecting cattle. This research has not yet reached a point of profitable summation.

G. Insect Vectors of Diseases

1. Anaplasmosis. Studies were continued in Texas, Mississippi, and Oregon to correlate the presence and abundance of insects and ticks with the incidence of anaplasmosis in herds of cattle. These studies were conducted in cooperation with the Animal Disease and Parasite Research Division and veterinarians of the various State experiment stations. In Texas, monthly surveys were continued to determine the abundance and identity of external parasites on infected (anaplasmosis) and clean herds of cattle. Lone star ticks were abundant on cattle during May, June, and July, 1962, but declined rapidly in August and partially disappeared by September. During this same time, ear tick populations were high except for August. Horn flies became numerous early in May but periodical spraying of cattle minimized populations the remainder of the season. The winter tick and black-legged ticks appeared in November. The winter tick was abundant by December, but black-legged tick populations remained low. Moderate populations of the ear tick were present from October through December. Small numbers of lone star and black-legged ticks and large numbers of ear and winter ticks were present on cattle in January, 1963. In February and March, 1963, populations of the lone star tick increased, while the winter and black-legged ticks virtually disappeared. Ear tick populations remained high.

Prophylactic treatment with aureomycin minimized transmission of anaplasmosis and prevented acute cases. All of the 10 animals in the control herd contracted the disease and 3 of them had acute cases. At the low level (2 mg.) the maximum number of negative animals occurred after 3 months of treatment, whereas at the high level the maximum number occurred 3 months after treatment was terminated. In time, most of the negatives reverted to positives. The period of greatest transmission of the disease coincided with that in which the horse flies, Tabanus lineola and T. vittiger schwardti, and the mosquito, Psorophora confinnis, were most abundant.

In Maryland, hereditary transmission experiments and histopathological studies with the vectors of anaplasmosis of cattle were continued in cooperation with the Animal Disease and Parasite Research Division. Two series of transmission trials with the vector, Dermacentor andersoni, were conducted

In one, the F_1 larval and adult progeny of female ticks fed on an animal in the acute stages of anaplasmosis failed to transmit anaplasmosis to test calves. In tests with larval progeny, challenge of the animals with the disease proved the animals to be susceptible to the disease. Proof of the susceptibility of animals used with adult progeny has not been completed. In another series of trials, unmated and mated males fed on an animal with acute anaplasmosis were studied for survival under hibernating conditions (4-5°C. and 30-50% relative humidity with a photoperiod of 7 hours) and their ability to transmit anaplasmosis. Mated males survived only about 4 months in the hibernating environment and transmission trials could not be run. Eleven of 49 unmated males survived the 8-month-hibernating period, and 6 of these survived for transmission trials 3 months after the hibernating period. None of the survivors transmitted anaplasmosis to test cattle; however, not all of the animals have been proved susceptible by challenge at this time. In similar studies previously reported, transmission by infected males was demonstrated.

In Maryland, histopathological studies on Anaplasma-infected and non-infected Dermacentor andersoni were continued. Using immunofluorescence methods, anaplasmata were found in gut and excreta smear preparations from ticks fed on infected animals. Brightfield and electron microscopic examinations revealed anaplasmata in excreta. Electron microscopic studies showed structures believed to be A. marginale in undigested erythrocytes. However, anaplasmata were not found in ultrathin section of the salivary glands and reproductive organs.

PUBLICATIONS REPORTING RESULTS OF USDA AND COOPERATIVE RESEARCH

Basic Biology, Physiology and Nutrition

Bigley, W. S. and Plapp, F. W. 1962. Metabolism of malathion and malaoxon by the mosquito Culex tarsalis Coq. Ins. Phys. 8: 545-57.

LaChance, L. E. and Leverich, A. P. 1962. Radiosensitivity of developing reproductive cells in female Callitroga hominivorax. Genetics 47: 721-35.

Harris, R. L. 1962. Laboratory colonization of the horn fly, Haematobia irritans (L.) Nature 196(4850): 191-2.

Eddy, G. W., Roth, A. R., and Plapp, F. W. 1962. Studies on the flight habits of some marked insects. Jour. Econ. Ent. 55: 603-7.

Lewis, L. F. and Eddy, G. W. 1962. The induced build-up of Bovicola bovis (L.) populations on cattle in Oregon. Jour. Econ. Ent. 55: 947-9.

Riley, J. and Hoffman, R. A. 1963. Observations on the meteorological-mosquito population relationship at Stoneville, Mississippi, 1959-60. Mosq. News 23: 36-40.

LaChance, L. E. and Bruns, S. B. 1963. Ovogenesis and radiosensitivity in Cochliomyia hominivorax (Diptera: Calliphoridae). Biol. Bull. 124: 65-83.

Insecticidal and Sanitation Control

Reed, L. B., Bushland, R. C., and Eddy, G. W. 1962. Systemic insecticides. 1962 Yearbook of Agriculture, pp: 340-3.

Roberts, R. H. 1962. Studies on the effect of radiation to pyrethrins applied to cattle. Jour. Econ. Ent. 55: 851-3.

Anthony, D. W. and Mott, L. O. 1963. Cattle lice eradication studies--a three-year evaluation. JAVMA 142: 130-2.

Bushland, R. C. and Radeleff, R. D. 1963. Development of systemic insecticides for pests of animals. Ann. Rev. of Ent. 8: 215-38.

Chapman, H. C. and Lewallen, L. L. 1963. Field tests with insecticides against mountain Aedes larvae in California. Mosq. News 23: 41-4.

Jones, C. M. and Medley, J. G. 1963. Control of the face fly on cattle with Co-ral in grain and on pasture. Jour. Econ. Ent. 56: 214-5.

Hoffman, R. A. and Roberts, R. H. 1963. Horn fly control studies in Mississippi, 1961. Jour. Econ. Ent. 56: 258-61.

Insecticide Residue Determinations

Claborn, H. V., Roberts, R. H., Mann, H. D., Bowman, M. C., Ivey, M. C., Weidenbach, C. P., and Radeleff, R. D. 1963. Residues in body tissues of livestock sprayed with Sevin or given Sevin in the diet. Jour. Agr. and Food Chem. 11: 74-6.

Derbyshire, J. C., and Murphy, R. T. 1962. Diazinon residues in treated silage and milk of cows fed powdered diazinon. Jour. Agr. and Food Chem. 10: 384-6.

Jackson, J. B., Radeleff, R. D., Roberts, R. H., Hunt, L. M. and Buck, W. B. 1962. Acute toxicity of Delnav and its residues in tissues of livestock. Jour. Econ. Ent. 55: 699-702.
Radeleff, R. D., Jackson, J. B., Buck, W. G., Younger, R. L., Weidenbach, C. P., and Roberts, R. H. 1963. Toxicity studies of Bayer 21/199 in livestock. Jour. Amer. Vet. Med. Assoc. 142: 624-31.
Radeleff, R. D., Jackson, J. B., Hunt, L. M., Buck, W. B., and Wrich, M. W. 1963. The acute toxicity of ronnel applied dermally to cattle and sheep. Jour. Econ. Ent. 56: 272-4.

Insect Sterility, Attractants and Other New Approaches to Control

LaBrecque, G. C., Meifert, D. W., and Gouck, H. K. 1963. Effectiveness of three 2-methyl-aziridine derivatives as house fly chemosterilants. Fla. Ent. 46: 7-10.
LaBrecque, G. C., Meifert, D. W., and Fye, R. L. 1963. A field study on the control of house flies with chemosterilant techniques. Jour. Econ. Ent. 56: 150-2.
LaBrecque, G. C., Smith, C. N., and Meifert, D. W. 1962. A field experiment in the control of house flies with chemosterilant baits. Jour. Econ. Ent. 55: 449-50.
Weidhaas, D. E. 1962. Chemical sterilization of mosquitoes. Nature 195 (4843): 786-7.
LaChance, L. E., and Knipling, E. F. 1962. Control of insect populations through genetic manipulations. Ann. Ent. Soc. of Ameria 55: 515-20.
Plapp, F. W., Jr., Bigley, W. S., Chapman, G. A., and Eddy, G. W. 1962. Metabolism of methaphoxide in mosquitoes, house flies, and mice. Jour. Econ. Ent. 55: 607-13.
Harris, R. L. 1962. Chemical induction of sterility in the stable fly. Jour. Econ. Ent. 55: 882-5.
LaChance, L. E. 1962. Effects of radiations on insects. Proc. North Cent. Br. ESA Meeting, XVII.
Graham, O. H. 1962. Research on chemosterilants for the control of livestock insects. Proc. North Cent. Br. ESA Meeting, XVII.

Insect Vectors of Diseases

Lindquist, A. W. 1962. Control of insect vectors of disease. Academic Press: 171-9.
Anthony, D. W. and Roby, T. O. 1962. Anaplasmosis transmission studies with Dermacentor variabilis (Say) and Dermacentor andersoni Stiles as experimental vectors. Proc. 4th Ann. Anaplasmosis Conf.: 78-81.
Hoffman, R. A. 1962. Control of vectors--equipment and insecticides. Proc. 4th Natl. Anaplasmosis Conf.: 84-5.
Roberts, R. H. 1962. Control of anaplasmosis through insect control, Washington County, Mississippi, 1961. Proc. 4th Natl. Anaplasmosis Conf.: 85-6.

AREA NO. 15. SHEEP AND GOAT INSECTS

Problem. Sheep and goats are attacked by a variety of insects and ticks that are responsible for losses of many millions of dollars annually in reduced weight gains, decreased production and quality of wool, and in deaths of animals from gross attacks and insect-borne diseases. Sheep keds are a particularly serious pest in the northern States and screw-worms in the southwestern States. Fleeceworms, lice, and ticks are important pests wherever sheep and goats are raised. Safer, more effective, nonresidue-forming insecticides are needed to combat these pests. There is a special need to develop systemic insecticides that when given at low levels in feed, salt, or water would effectively control pests of sheep and goats and thereby save growers the expense of rounding up and treating flocks several times a year. New approaches to control, including attractants, chemosterilants, and radiation, should be explored and developed for controlling certain pests, as was done for the screw-worm in the Southeast. The possibilities of controlling insect pests of sheep and goats with insect pathogens, parasites, and predators also need to be investigated. Additional basic studies on the biology of the insects involved are essential for the development of biological and sanitation measures for their control. Research is urgently needed to determine which insects other than sand flies transmit bluetongue and the role of insects and ticks in the spread of other diseases of sheep and goats.

USDA PROGRAM

The Department has a continuing program involving basic and applied research on insects and ticks which affect the health and productivity of sheep and goats. Studies are conducted on the biology, physiology, and nutrition of pests of sheep and goats, particularly the screw-worm and Culicoides gnats, with some attention to sheep keds and lice; on the nature of resistance to insecticides and on the length of time insecticides remain on animal skin and hair; and on the absorption, metabolism, degradation, excretion, and mechanism of action of insecticides on the insects. A program is underway to find new ways to control pests of sheep and goats, with special emphasis on chemosterilants, antimetabolites, attractants, and non-insecticidal materials. Efforts are being made to develop adult screw-worm attractants for determining the abundance of natural populations and for use in baits for control. Research is concerned with the development of more effective contact and systemic insecticides and with studies to devise sanitation or management procedures to minimize or prevent insect reproduction. Primary emphasis is given to the evaluation of new materials that leave small amounts of or no residues and to testing of formulations that will prolong effectiveness against insects and minimize toxicity hazards. Studies are conducted to determine the occurrence of residues

in tissues of animals treated with insecticides in cooperation with the Animal Disease and Parasite Research Division. A limited program is being conducted on the relationship of insects to diseases of sheep and goats, involving experimental transmission from diseased to healthy animals with various species of insects, and insect surveys in epidemic areas. Current studies are centered on the insect vectors of bluetongue disease of sheep. This work is conducted in cooperation with the Animal Disease and Parasite Research Division. The research is conducted in major laboratories in Kerrville, Tex., and Corvallis, Oreg., and in satellite laboratories in Mission, Tex., and Denver, Colo. Investigations on the screw-worm were discontinued at Kerrville in September 1962 and moved to Mission, Tex., headquarters of the Southwest screw-worm eradication campaign. At the beginning of FY 1963 the bluetongue transmission research was transferred from Kerrville, Tex., to Denver, Colo.

The Federal scientific effort devoted to research in this area totals 4.9 professional man-years. Of this number, 1.9 is devoted to basic biology, physiology, and nutrition; 1.4 to insecticidal and sanitation control; 0.4 to insecticide residue determinations; 0.4 to insect sterility, attractants, and other new approaches to control; 0.6 to insect vectors of diseases; and 0.2 to program leadership.

REPORT OF PROGRESS FOR USDA AND COOPERATIVE PROGRAMS

A. Basic Biology, Physiology and Nutrition

1. Screw-worm. Investigations included research on the biology and ecology of the screw-worm under field conditions, vigor and longevity of sterilized flies, effects of radiation, development of genetically-marked strains, cytological studies with irradiated flies, nutrition, and special tests related to problems in the sterile male release program.

A new technique to determine the mating aggressiveness of sterilized or mutant screw-worm flies was developed. In laboratory cages males harassed females sufficiently to cause mortality greater than that occurring when females were caged without males - in general, higher ratios of males to females caused greater mortality than lower ratios. Tests with various strains of screw-worms of known sexual competetiveness in comparison with other mating aggressiveness tests confirmed the validity of the method. Since the criterion for mating aggressiveness is the mortality of females over time rather than egg production and viability, the time, space, and labor required to determine sexual aggressiveness were greatly reduced.

Studies confirmed optimum numbers of screw-worm larvae per tray for rearing efficiency and optimum numbers of adults per cage for mating and longevity studies. Diurnal periodicity in screw-worm pupae for

emergence occurred from sunrise until noon. Research has shown that the use of CO_2 as anesthesia for screw-worms is safe for handling young screw-worm adults for longevity and mating tests and scanning for genetic markers.

Research was continued to find and develop genetically distinct strains of screw-worm flies and to study these mutant flies. In the adults many genetic markers were found such as yellow eyes and white auxiliary region ("whaxy"). The genetic marker "whaxy" affects the morphology of both the adult and larva--a factor which would be invaluable in field studies. Approximately 270,000 screw-worm larvae were examined for morphological variants and from this 221 cultures were studied as possible genetic strains. Of markers found in the immature forms, at present three morphological characters--interrupted bands of segmental spines, two spiracular openings in the anal plates of the third-instar larva, and additional spines on the eleventh anterior segmental band--were demonstrated to be genetic in nature. None of these were established as pure strains. The genetics of these and other mutant strains are being determined. The mutant strains must be as vigorous and aggressive as the normal strain if they are to be used in the release program. For example, preliminary genetic studies indicate that the "whaxy" mutant is lethal in the homozygous state with only a few individuals escaping. Other strains appear to have slight behavioral differences from the normal strain, i.e., they responded differently to attractants and survey traps.

When pupae of the screw-worm were irradiated in an atmosphere of CO_2 and air (50-50 mixture), damage to the reproductive system of the adult female was greater than that induced by a similar radiation treatment in air. When the pupae were pretreated in the gas mixture for approximately 45 minutes, complete sterility was induced by a treatment of 4,500 r delivered in CO_2 and air whereas irradiation in air alone required about 5,500 to 6,200 r.

Techniques were developed to study the cytological effects of radiation and chemosterilants on the screw-worm, particularly on spermatogenesis and oogenesis. Preliminary studies revealed chromosomal aberrations severe enough to cause dominant lethality. Considerable data on oogenesis and spermatogenesis in normal screw-worm flies were accumulated as necessary background material for evaluating the effects of radiation and chemosterilants on reproduction.

For rearing larvae of the screw-worm, a synthetic diet was developed that contained casein, yeast extract, cholesterol, inorganic salts, water, and agar. This medium was further defined by replacement of casein with a mixture of L-isomer amino acids, and of yeast extract with a mixture of B-vitamin and RNA. Larval growth and development on the defined medium were nearly equal to that on media containing casein

and yeast. Larvae absolutely required thiamine, riboflavin, pantothenate, niacin, and choline for growth. Biotin and folic acid stimulated growth and were necessary for maturation to the adult stage. Pyridoxine and its analogs, pyridoxal and pyridoxamine, inositol, B_{12}, and carnitine had no effect on growth. Niacinamide replaced niacin, but p-aminobenzoic acid had no effect on the folic acid requirement.

Studies on the effect of dessication, lack of adult food, reduced temperature, age of flies at time of release, and the effect of all of these factors, on different strains of the screw-worm were undertaken to improve the survival of screw-worm flies released into the hot and dry climate of Texas. Provision of food in release cartons or the judicious use of reduced holding temperatures curtailed mortality prior to or shortly after release. Techniques need to be developed which would allow greater uniformity in age of flies at the time of release. Newly emerged flies carry a food and water reserve that will sustain them up to 24 hours at 90° F. and 30% R.H., compared to only 3 to 4 hours for 2-day-old flies under the same conditions. Selection of flies for individuals more capable of survival under unfavorable conditions has shown promising results.

Extensive studies were made on the ecology of screw-worm flies under field conditions by releasing tagged flies. The flies tended to congregate and disperse along water courses or streams and were capable of travelling distances up to 180 miles in 5 to 11 days. This finding resulted in the addition of strategic releases of screw-worms along water courses and an increase in the effectiveness of the sterile-male release program.

2. Lice. Three species of lice were found on Texas goats in 11 counties, Bovicola limbata (the angora goat biting louse), B. caprae (the goat biting louse), and B. crassipes (the hairy goat louse). These lice were collected from January to April.

Fluctuation in numbers of Bovicola limbata was studied on Angora goats ranging from 4 to 7 years in age. In general, goat herds were lightly infested during the summer and lice did not increase appreciably until fall. The weather was extremely hot and dry from mid-July to September, and the time spent in various immature stages by the lice was prolonged. Thus, high temperatures and low humidities may decrease the lice populations by reducing the number of female lice and lowering egg production and also by prolonging the development of the lice from the egg to the adult stage. The over-all pattern of fluctuation in lice numbers showed a progressive increase after each shearing, although there was a drastic reduction in the number of lice after each shearing, with the greatest reduction in male lice. Lice populations continued lower through 2 or 3 weeks after shearing. Mohair growth averaged slightly over 0.8 inch per month. The number of lice on certain goats declined prior to each shearing and the host-parasite

relationship appeared to be the most important factor determining the number of lice on an animal. For example, on December 1, one goat was in such poor physical condition that it was treated intramuscularly with 20,000 units of penicillin; during January it was isolated from the other goats and fed grain supplement, as well as the standard alfalfa ration. In February, the goat became active and was returned to the pen. Concurrent with the physical improvement, the number of lice declined and continued low. However, on July 13, the sex ratio (females:males) of the lice, usually 2:1 to 5:1, was reversed. The same situation was found on another goat on July 20. Two other goats had moderate numbers of lice in the winter and the numbers were declining prior to the spring shearing. However, the lice increased at the beginning of the hot dry summer weather and were present in high numbers at the fall shearing. The increase in numbers of lice on a few low-resistance goats may account for the rapid spreading of lice and heavy infestation of the herd usually observed in the fall.

Off-the-host studies with Bovicula limbata, held at 95° F. and 75% relative humidity, in a constant temperature cabinet showed the following development times: average number of days in the first instar, 6.3 (range, 5-8 days); second instar, 4.9 (range, 4-6); third instar, 6.6 (range, 5-11). For an adult life averaging 15.5 days (range, 7-29 days), the preoviposition period was 3.5 days (range, 2-5 days). The number of eggs per female averaged 7.6 (range, 2-17). The number of days in the egg stage was 8.4 (range, 7-9 days). In a more limited study on the length of development time in the goat sucking louse, Linognathus stenopsis, the average length of the three instars was 14.5 days. These off-the-host studies were conducted in cooperation with M. A. Price of Texas A. and M. College.

3. Biting Flies and Gnats. Tabanids (horse flies and deer flies), snipe flies, and black flies were serious pests of sheep and other animals in the Coast Range of Oregon. There were several hundred black flies per head, along with smaller numbers of snipe flies and tabanids. For daytime protection from these pests, domestic stock was forced to take shelter and remain quiet in dense stands of timber, or to remain quiet in full sunlight in the middle of open pasture. Entomologists collected 366 blood-sucking flies in one hour on a restrained horse. Little is known about the biology of the snipe flies; a few adults have been collected in emergence traps placed over likely larval sites. A few snipe fly larvae were collected in the spring of 1963. It is not yet known with certainty whether these are Symphoromyia--the genus found annoying as adults on the stock.

B. Insecticidal and Sanitation Control

1. Sheep Nose Bots. In Texas, six yearling lambs were treated with a commercially available Ruelene drench. Three were treated at 100 mg./kg. and three at 170 mg./kg. Six other lambs were left untreated for controls. All animals were sacrificed and their nasal chambers and sinuses

examined for bots. The six untreated lambs had 543 first-instar bots, 13 second-instar bots, and 4 third-instar bots, an average of 93 bots per lamb. The higher dosage of Ruelene killed all bots of all instars, but the lower dosage permitted survival of one first- and one second-instar bot.

2. Lice. In Texas, a spray containing 0.25% of Dilan (1 quart per head at 250 p.s.i.) controlled biting lice, Bovicola crassipes and B. sp., on a flock of Spanish goats, but failed to control sucking lice, Linognathus stenopsis. In Angora goat flocks, Ruelene at 0.25%, diazinon at 0.05%, and Ciodrin at 0.25%, gave excellent control of biting lice, B. limbata. Ruelene also controlled sucking lice, L. stenopsis, but the sucking lice reappeared on the goats treated with Ciodrin after 2 months. Ciodrin at 0.1% did not give satisfactory control of either biting or sucking lice.

Incidental to cooperative studies with veterinarians of the Animal Disease and Parasite Research Division to determine toxicity of some dips to goats, observations were made on the effectiveness of Zectran at 0.25%, Geigy 30492 at 0.1%, and Stauffer R-1504 at 0.5%. All dips were effective against both biting and sucking lice and none of the goats became reinfested through 1 month, at which time the tests were terminated.

Angora goats moderately to heavily infested with biting lice, Bovicola limbata and sucking lice, Linognathus stenopsis, were dipped in various concentrations of Sevin, with and without the addition of the synergist, sulfoxide. The addition of the sulfoxide increased the residual effectiveness of Sevin, as lice were not found on the goats dipped in the combination (0.05% Sevin, 0.05% sulfoxide) until 42 days after dipping. Goats dipped in this concentration of Sevin without the synergist showed lice 7 days after dipping. Tests at various concentrations indicated greater effectiveness against sucking lice than against biting lice. Sevin alone was ineffective against both kinds of lice below a concentration of 0.15%. With an equal concentration of sulfoxide, treatments below 0.1% of Sevin were ineffective against biting lice and treatments below 0.025% Sevin were ineffective against sucking lice.

In Oregon, one hundred milliliters per animal of 2% toxaphene, 1% Famophos, 0.3% Baytex, or 1% Ruelene, were poured along the backs of Angora goats for control of goat lice. Baytex was also applied to one group of goats in a spray at 0.1%. After 3 weeks all pour-on treatments were about equally effective, with the control ranging from 96.4% to 97.6%. The Baytex spray gave 99.5% control.

3. Ticks and Keds. Early in the summer in Texas, three flocks of sheep were found infested with the sheep ked. The older animals were lightly infested, but large numbers of keds were found on the older lambs. One flock of 230 sheep was sprayed with 0.1% General Chemical GC-4072;

another flock of 284 sheep was sprayed with this insecticide at 0.25%. The remaining flock of 120 sheep was sprayed with 0.25% V-C 13. Pretreatment counts of keds ranged from 13 to 123 per lamb in the three flocks. The flock treated with V-C 13 showed complete control 1 day after treatment. GC-4072 was slower in action, the 0.25% spray showing only 33% control 1 day after treatment, but 100% control of the keds 1 week after treatment. The 0.1% spray gave no measurable control the day after treatment, only 14% 1 week after treatment, and only 25% 2 weeks after treatment. However, after 1 month, complete control of keds was indicated.

In Oregon, a small flock of sheep with a light infestation of sheep ked (average 8/head) was treated with one gram of technical barthrin per head. The insecticide was applied as a 5% solution in corn oil with a pump oil can at the rate of 20 ml. (2/3 fluid ounce) per head. Control was excellent (97.5%) with only one live ked remaining after 3 weeks.

C. Insecticide Residue Determinations

1. Toxicology Studies. Work was conducted in Texas in cooperation with veterinarians of the Animal Disease and Parasite Research Division on the acute and chronic toxicity of insecticides and other materials. A summary of the results is presented. Detailed results will be given under Unit 2, Animal Diseases and Parasites, ADP a7-19.

A colorimetric analytical method for studying the toxicology and presence in animal tissues of 2,4-D was successfully developed by using carbon-14 labeled 2,4-D which could be determined with radiation detection instruments at each step of the developing technique. The analytical method which resulted is capable of detecting 0.05 p.p.m. of 2,4-D in animal tissue samples weighing 25 grams.

In studies of the detoxication mechanisms in cattle and sheep, oximes, including 2-PAM chloride, DAM, and P_2S, were used to counteract poisoning by various organophosphorus compounds. The oximes cause a release of the enzyme (cholinesterase) inhibited by this group of pesticides. All three compounds were effective in mild or moderate poisoning. Their action was somewhat slow and atropine sulfate was still required in severe poisoning to gain time for the oximes to work.

Sodium selenite, sodium selenate, and d-alpha tocopheryl (Vitamin E) were effective in several instances of organophosphorus poisoning. Studies are needed to explore the mechanisms by which these two substances exert their beneficial effects.

Studies of lindane sheep dips revealed that improper physical formulations were being employed, permitting the first sheep dipped in a fresh vat to so deplete the fluid that the sheep dipped later were

receiving dangerously small concentrations insofar as control of parasites such as the scabies mites was concerned. Because of the excessive amounts taken out by the first sheep dipped, these animals developed tissue residues far in excess of those normally to be expected.

Residues of 2,4-D in sheep fed the compound at a rate of 2 grams per head per day at Logan, Utah, were determined at Kerrville, using an analytical technique developed by the Kerrville staff. In sheep fed 30 daily doses, kidney, rumen, renal fat and body fat samples showed less apparent residue than did a control sheep. Muscle samples averaged less than 0.3 p.p.m. and liver samples less than 1.0 p.p.m. of 2,4-D.

In animals killed by the insect chemosterilants apholate, aphoxide, and methaphoxide, cytological changes were most prominent in the organs engaged in formation of the white cells of the blood. The changes indicated a severe decrease in ability of the parent tissue to supply the needs of the animals. In other studies, these materials were highly cumulative in effect. Although some studies using low-level feeding are still current and the animals still alive, every dosage thus far tried has ultimately killed every sheep treated, in some cases after one year or more of exposure during which no observable illness occurred.

Thirty-two insecticides, most of them currently under test against livestock insects, were studied. These studies furnished toxicological guidelines for decisions for further development of the materials.

D. Insect Sterility, Attractants, and Other New Approaches to Control

1. Screw-worm. In Texas where studies on the screw-worm were conducted, 57 of 350 compounds screened as candidate chemosterilants caused sterility when administered as topical treatments or fed to adults. Some of the compounds sterilized by both methods of administration. Some of these compounds sterilized one or both sexes completely while others induced only partial sterilization or were ineffective. Tests with tretamine applied topically showed that adult flies could be sterilized when they were 1, 3, and 5 days old with equal facility. Males sterilized with thiotepa survived as well as untreated flies and competed equally with untreated males in mating with females. However, the treated males were not as sexually competitive as untreated males. When a single dose of thiotepa or tretamine, adequate to sterilize either sex, was given in two half-doses 24 hours apart, survival of the flies was not improved and, further, a loss in sterilizing effectiveness occurred. Tretamine and thiotepa completely or partially sterilized screw-worms when puparia were immersed in solutions containing these compounds or were injected with them. With immersion, washing of puparia decreased the sterilizing effect, indicating that the adult obtained some or most of the sterilizing dose as it emerged from the puparium. Aerosol treatment of screw-worm adults with tretamine resulted in almost complete sterility.

Laboratory experiments indicated that ENT-50450 was as effective as gamma radiation in sterilizing screw-worm flies and was superior in its lack of toxic side effects. Females mated to males sterilized with ENT-50450 continued to lay infertile eggs after the initial deposition of eggs indicating that sperm in the spermathecae of females did not recover fertility. Males sterilized with ENT-50450 remained sterile throughout their lifetime.

Investigations with screw-worm flies and aziridinyl-type chemosterilants resulted in the conclusion that the primary influence of aziridinyl compounds on the ovaries of flies 0-4 hours old is the inhibition of oogensis, and of flies 1 day old, the induction of mutations. The effects of the aziridinyl compounds on the reproductive potential of female screw-worm flies were similar to those obtained with gamma radiation.

Studies were conducted to determine the absorption, metabolism, and excretion of a P^{32}-labeled chemosterilant (metepa) applied topically and in the diet of screw-worm flies. When applied topically the material was absorbed rather gradually. The material was metabolized to the extent of 42 and 58% in males and females, respectively, in 24 hours. The principal metabolic products were phosphoric acid and an unknown intermediate. Small amounts of five other intermediates were indicated but were not identified.

In the feeding tests, metepa was absorbed and distributed at about the same rates as in the topical tests. After 24 hours, the degree of metabolism in females was about the same as that in the topical test but, in the males, metabolism was 50% higher than in the topical tests. As in the feeding tests, the main metabolic products were phosphoric acid and an unknown intermediate. Five other intermediates were isolated but not identified. Approximately one-fifth of the applied dose of metepa was excreted.

Approximately 200 chemicals and other materials were screened as attractants for screw-worm flies. Of these, 10 were equal to or better than the standard liver bait and require further evaluation. Mutant strains of screw-worm flies may respond differently than normal strains to attractants since black-mutant flies were not attracted to the standard liver bait.

2. Ticks. Extensive tests were conducted in Texas to determine the effects of different levels of radiation on different stages of the lone star tick. One series of tests with nymphs which had been engorged 2 weeks were exposed to different levels of radiation and the adults subsequently placed on hosts. Adults from nymphs exposed to 500 r or 1000 r engorged but produced no eggs. At a dose of 2500 r adults engorged normally and treated females mated with treated males did not oviposit. One of 8 treated females mated with normal males

oviposited and some of the eggs hatched. When females and males treated with 5000 r were confined on hosts neither sex engorged. Similar results were obtained with untreated males and treated females, but when treated males and untreated females were used 2 of 3 females engorged and oviposited. One of the egg masses was not viable and the other showed only a partial hatch.

Exposure of newly emerged adult ticks to 1000 r and 2500 r did not affect engorgement and complete sterility was indicated in crosses of treated males and females and in crosses of untreated males and treated females. Untreated females mated with treated males produced eggs but none hatched. One of the ticks exposed to 5000 r engorged but it did not oviposit. No ticks engorged after treatment with dosages of 7500 r or 10,000 r.

Some of the adult ticks dipped in concentrations of 0.25 and 0.5 percent of apholate, tepa, metepa, or tretamine were killed, but those that survived engorged and laid viable eggs. Dipping in 1% concentrations of these materials also failed to produce complete sterility but the maximum viability of eggs from females treated with apholate was only 0.25%.

E. Insect Vectors of Diseases

1. Biting Flies and Gnats. Studies were continued in cooperation with the Denver, Colo., laboratory of the Animal Disease and Parasite Research Division, on the transmission of bluetongue disease of sheep. The colony of Culicoides variipennis was less satisfactory than it had been at Kerrville, Tex., requiring studies to improve culture techniques. Techniques were developed for large scale feeding of the insects on embryonating hen's eggs and on various fluids through membranes. An attempt was begun to culture bluetongue virus in embryonating hens eggs. The Culicoides fed readily on allantoic fluid of the eggs. Insects were collected, but are not yet fully identified, from an area of bluetongue outbreak in Montana, Wyoming, and South Dakota.

PUBLICATIONS REPORTING RESULTS OF USDA AND COOPERATIVE RESEARCH

Basic Biology, Physiology, and Nutrition

Jones, R. H. 1962. Rearing records for Culicoides loughnani Edwards (Diptera, Ceratopogonidae). Annals Ent. Soc. of America 55(6):721-2.

LaChance, Leo E. and Ann P. Leverich. 1962. Radiosensitivity of developing reproductive cells in female Callitroga hominivorax. Genetics 47: 721-35.

LaChance, L. E. and Sarah B. Bruns. 1963. Ovogenesis and radiosensitivity in Cochliomyia hominivorax. Biol. Bull. 124: 65-83.

Insecticidal and Sanitation Control

Bushland, R. C. and R. D. Radeleff. 1963. Development of systemic insecticides for pests of animals. Ann. Rev. of Ent. 8: 215-38.

Harris, R. L. and R. H. Jones. 1962. Larvicide tests with colony-reared Culicoides variipennis. Jour. Econ. Ent. 55(4): 575-6.

Reed, L. B., R. C. Bushland, and G. W. Eddy. 1962. Systemic insecticides. 1962 Yearbook of Agriculture, pp: 340-43.

Insect Sterility, Attractants, and Other New Approaches to Control.

Graham, O. H. 1962. Research on chemosterilants for the control of livestock insects. Proc. North Cent. Br. ESA Meeting, Vol. XVII.

LaChance, Leo E. 1962. Effects of radiations on insects. Proc. North Cent. Br. ESA Meeting, Vol. XVII.

AREA NO. 16. POULTRY INSECTS

Problem. Numerous species of insects, mites, and ticks are common pests of poultry throughout the country and if not controlled can make poultry raising unprofitable. They cause poultry to look unsightly, reduce weight gains and egg production, and mar the skin, which results in downgrading of quality and lower market prices. Pests such as black flies and mosquitoes transmit leucocytozoon and fowl pox diseases which exact a heavy toll in death and unthrifty poultry each year. House flies spread parasites and enteric diseases which may decimate flocks. Safer, more effective nonresidue-forming insecticides are needed to combat these poultry pests and vectors of diseases of poultry. Better materials are needed for direct application to poultry or in poultry houses to control lice, mites, and ticks and for use as larvicides or fly baits to control flies. Materials are especially needed which, when given in feed or water, would act systemically to control external pests and render droppings toxic to fly larvae. Exploratory studies are needed to investigate possibilities of developing attractants, chemosterilants, antimetabolites, or other new methods of combatting poultry pests. Biological and sanitation methods of control offer excellent possibilities for control and need to be emphasized. There is a special need to investigate the roles of insects, ticks, and mites in the transmission of poultry diseases.

USDA PROGRAM

A continuing study is underway involving basic and applied research on insects, mites, and ticks that affect the health and productivity of poultry. Studies are designed to determine breeding habits and reproductive capacities of various poultry pests and to gain further knowledge on the nature of resistance of these pests to certain insecticides. Work at present is devoted mostly to lice and the northern fowl mite, and to the house fly, which breeds abundantly in poultry droppings. A newly expanded program aims to find new ways to control pests of poultry with special emphasis on chemosterilants, antimetabolites, attractants, and noninsecticidal materials and methods. Current studies in this field are largely limited to house flies. They include investigations of physical and mechanical methods for controlling house flies being conducted in cooperation with the Agricultural Engineering and Animal Husbandry Research Divisions.

Research is concerned with the development of more effective insecticides for the control of poultry pests. New chemicals are screened for contact and residual toxicity to lice and mites attacking poultry and to house flies, and promising ones are tested for effectiveness under practical field conditions. New methods of utilizing insecticides more efficiently and safely are being investigated, with special attention to finding materials that, when given orally in water or feed, will act systemically to kill lice and

mites on the poultry, and render the droppings toxic to fly larvae. Efforts are also being given to methods of sanitation and management to control fly breeding in accumulations of manure in poultry houses. Studies are conducted to determine the occurrences of residues in tissues of poultry treated with insecticides. Work is done in cooperation with poultry raisers at Orlando, Fla., Stoneville, Miss., Corvallis, Oreg., and Kerrville, Tex. Additional research is conducted at Fresno, Calif., and Beltsville, Md.

The Federal scientific effort devoted to research in this area totals 2.2 professional man-years. Of this number, 0.4 is devoted to basic biology, physiology, and nutrition; 0.9 to insecticidal and sanitation control; 0.2 to insecticide residue determination; 0.6 to insect sterility, attractants and other new approaches to control; and 0.1 to program leadership.

REPORT OF PROGRESS FOR USDA AND COOPERATIVE PROGRAMS

A. Basic Biology and Physiology

1. House Flies. At Orlando, Fla., research on control or eradication through the use of sterilization by radiation or chemicals has shown that considerable gaps exist in knowledge of the biology and mating behavior of house flies. Both males and females must undergo a sexual maturation time of at least 16 hours with males and a minimum of 24 hours with females. Once the mating drive has started, males will attempt to "strike" or mate with both males and females and certain inanimate objects, although they "strike" more readily and frequently with females. This fact along with experiments in a large cage-type chemotactometer suggested the presence of some type of a female sex attractant of a low order. Imperfect sex recognition in the male combined with a low order sex attractant in the female would account for the fact that males attempt to mate with either sex and result in the higher ratio of male-to-female "strikes." Males or females which had their wings removed were able to mate with individuals of the opposite sex with wings. Amputation of more than one pair of legs from the male prevented mating, whereas amputation of only one pair of legs impeded but did not prevent mating.

Tests were run to determine the actual time house flies remain in copula after initial seizure. Of 61 mating pairs that were trapped and observed, the shortest mating period was 44 minutes and the longest, 96 minutes. The average was 60 minutes. After mating periods of only 1 and 2 minutes, no sperm were found in females, but after 3 to 5 minutes some spermathecae contained a few sperm and some were completely filled. For periods of 10 to 76 minutes spermathecae were filled to capacity with sperm with only one exception.

Observations on the natural sterility of 86 female house flies reared in the laboratory gave an average of 94.4 eggs per female of which 89% hatched. The number of eggs laid varied from 12 to 186 per female.

Investigations to determine how soon male and female house flies fed after emergence showed that some flies fed within an hour after emergence, others did not feed until later, and some did not feed until they were 16 to 18 hours old.

At Corvallis, Oreg., studies were continued on the physiology of resistant and susceptible house flies. Experiments have shown that Isolan-resistant house flies have decreased ali-esterase activity and increased Isolan-detoxifying enzyme. Techniques using high-speed centrifugation have been developed which concentrate enzymes important in metabolizing or conferring resistance to organophosphorus compounds. Experiments with malathion-resistant house flies indicate that the nature of the alkyl groups attached directly to the phosphorus atom is the major factor in resistance. Thus, the primary cause of resistance is probably associated with the rate of recovery of the phosphorylated detoxifying enzyme after poisoning, rather than to an increased ability to cleave the toxic molecule per se.

In Oregon extensive studies were conducted on the biology, nutrition, and colonization of the little house fly (Fannia canicularis). After much trial and error a satisfactory rearing medium was developed which consisted of alfalfa meal, yeast extract solution and wood shavings. Adult flies are held in standard cages. Wrinkled balls of black muslin impregnated with alfalfa and yeast extracts are provided as oviposition sites. Forty-eight percent of the females and 25% of the males survived for 22 days in cages kept at 70-80°F. At 90°F. no males and only 8% of the females survived for 22 days. Egg deposition was about 5 times as great at 80°F. as at 70° or 90°F. Females began ovipositing in 5-6 days at 90°F., 8-9 days at 80°F. and 10-11 days at 70°F. The eggs dessicate rapidly and must be kept constantly on a moist surface to insure a high percentage of hatch.

2. Mosquitoes. At Corvallis, Oreg., and Fresno, Calif., studies were continued on the biology and ecology of important mosquito species. In laboratory tests the majority of Culex tarsalis females preferred to feed at the higher of two feeding locations and preferred mice rather than chickens for their source of blood.

A study was conducted on the daily activity of several species of mosquitoes --Anopheles freeborni, Culex peus, Culiseta inornata, Culex incidens, and Culex tarsalis. All species behaved similarly. Both males and females began to move out of daytime resting shelters a few minutes before or after sunset. Only a few males and females (0.3 to 2.8%) remained in the shelters during the night. Adults did not begin to return to the shelters until sunrise or a few minutes later, and all did not return until sometime after 8:00 a.m. Exodus from and return to shelters appeared to be regulated by light intensity, but some other factors such as preconditioning to light cycles, temperature, and humidity may have had some effect since artificial lights did not prevent mosquitoes from leaving shelters eventually, but did change their time and rate of leaving. Use of special crosses of resistant

and susceptible strains of Culex tarsalis showed that some of the females mate more than one time and utilize sperm from more than one mating.

Taxonomic studies in California have defined suitable characters for separating dark-winged forms of Aedes dorsalis from A. melanimon and have shown that these two forms coexist in only one area (Solano County). Surveys at Borrego Springs (San Diego County) were made to find isolated populations of mosquitoes for future studies on control through sterilization.

Surveys were continued on biting arthropods of the Humboldt River Basin in Nevada. During April, May, and June, water was plentiful and high populations of Aedes melanimon, A. dorsalis, A. vexans, Culex tarsalis, and Culiseta inornata were found. Mosquito larvae were infected with a microsporidian, Thelohania sp. and a bacterium, both of which are under study as biological control agents. In late July, no floodwater Aedes breeding was observed, but moderate breeding of Culex tarsalis and Culiseta inornata was observed. Studies of mosquito larval populations in typical breeding areas in dairy drains and culverts in California during April indicated the presence of Culiseta inornata, Culiseta incidens, Culex tarsalis, Culex peus, Culex apicalis, and Anopheles franciscanus, but no breeding of Culex quinquefasciatus was found.

Using C-14 labeled TDE, slight but consistent differences in its rate of degradation have been noted in Oregon in susceptible and resistant strains of Culex tarsalis indicating that the main source of resistance to this chemical is increased degradation of TDE to FW-152 and water soluble metabolites through an oxidative rather than a dehydrochlorination mechanism. Further tests with DDT and TDE have shown that analogs of DDT, which block metabolism via the oxidative pathway, overcome resistance to DDT and tend to confirm the hypothesis that resistance to DDT and TDE in tarsalis is a more rapid oxidative metabolism of the insecticide.

B. Insecticidal and Sanitation Control

1. House Fly. Research was continued at Orlando, Fla., to develop more effective insecticides and other methods and materials for the control of house flies. Twenty-three new compounds were tested as space sprays in a wind tunnel against the regular susceptible colony and the Cradson (multiresistant) colony. Fourteen of the compounds were more effective against both the susceptible and resistant colonies than the standard, malathion. New insecticides were also evaluated as residual treatments against female house flies from the regular or Cradson colonies. The criterion of effectiveness was the number of weeks of aging during which the residues remained effective in killing house fly females. Against susceptible house flies Hercules AC-5727 alone and Bayer 39007 alone gave kills of 90% for one week, but in combination with Monsanto CP-16226, their effective periods were extended to 8 weeks and 12 weeks, respectively. The

effectiveness of Hercules 7522H was also extended from 1 to 12 weeks with the addition of Monsanto CP-16226. Against flies of the Cradson colony all of these formulations failed before the fourth week. Against the regular colony, Bayer 29952 and Bayer 30237 were effective for 64 and 56 weeks. Stauffer N-2230 and Stauffer N-2404 were 100% effective throughout 48 weeks of aging against the susceptible colony, but they were ineffective against the Cradson colony. General Chemical GC-3583 was still 100% effective after 96 weeks against the regular colony and Monsanto CP-40294 was effective for 24 weeks against the Cradson colony. Against house flies from the susceptible colony, General Chemical GC-4072 was 100% effective for 96 weeks as an acetone solution and Stauffer N-2310, Bayer 39197 and Monsanto CP-40273 for 48+ weeks. As wettable powders, DDT was more than 90% effective for 48 weeks, Bayer 25141 for 48 weeks, Bayer 34098 for 40 weeks, Hooker HRS-1422 for 32 weeks, and Bayer 32651 for 28 weeks.

Residual tests (deposits of 100 mg./sq. ft.) were conducted with emulsions of diazinon, Baytex, and dimethoate against house flies in Florida dairy barns. The diazinon treatment failed to give satisfactory control as early as the first day after treatment. Baytex gave 97% control for 5 days and from 79% to 88% control through 14 days. Dimethoate gave controls ranging from 80% to 96% for 6 weeks, when the test was discontinued.

At Corvallis, Oreg., extensive studies were continued on the development of synergists that have overcome resistance to organophosphorus insecticides in both house flies and mosquitoes. Of some new types of compounds screened, results indicate that diisopropyl or dibutyl analogs would be most satisfactory. Selection of house flies with combinations of malathion and synergists are being carried out to determine if resistance to the combinations can be developed.

2. Mosquitoes. Studies were continued at Orlando, Fla., to find new and effective insecticides for the control of mosquitoes. In screening tests with Anopheles quadrimaculatus larvae, 28 of 81 compounds tested were rated Class IV in effectiveness. Four of these compounds--American Cyanamid CL-18133, Stauffer B-10046, Stauffer 8-10094, and Bayer 47940--were highly effective, killing 100% of the larvae at concentrations of 0.05 p.p.m. to 0.01 p.p.m. One hundred and nine plant extracts were also screened for toxicity, but none of these were toxic at low concentrations. Of 83 compounds tested against female Aedes taeniorhynchus in wind tunnel tests, 7 were as effective as the standard, malathion. Dimethrin in granular formulations was effective in laboratory and field tests as a larvicide against Anopheles quadrimaculatus, Aedes aegypti, Aedes taeniorhynchus and Culex quinquefasciatus mosquitoes. A mixture of DDT and anti-resistant compound was no more effective than DDT alone against C. quinquefasciatus larvae.

In Oregon, 46 compounds were tested for systemic action by giving them orally to mice and allowing mosquitoes to feed on the mice. At a dosage of 100 mg./kg. two compounds--Bayer 29493 and Shell SD 8436--killed all mosquitoes for 6 hours after the mice had been treated.

3. Lice and Mites. Nineteen chemicals were tested in Texas for control of poultry lice on chickens. All but two chemicals controlled all species of lice for at least 3 weeks at a level of 0.1% in sprays; 15 chemicals were fully effective for 3 weeks at 0.05%, and 3 chemicals were effective at 0.025%. All treatments were applied as sprays directly to the individual birds.

White leghorn hens having body lice (Eomenacanthus stramineus) and wing lice (Lipeurus caponis) were sprayed or dipped in Dowco-175 or Ciodrin. Each bird was treated individually. In the Dowco-175 tests, both full dips and 40 ml./bird sprays killed all lice within 3 days at concentrations of 0.05% to 1.0%. The Ciodrin tests were conducted to compare the effectiveness of 0.05% and 0.1% in sprays, 0.05% in full dips, and 0.05% in dips in which only the tail of the bird was immersed. All Ciodrin treatments eliminated lice prior to 10 days post-treatment. In all these tests, after the initial 10 days of isolation, the treated birds were placed together in a henhouse with 4 untreated birds in an attempt to either reinfest the treated birds, or decrease the population of lice on the untreated birds. No change occurred on either throughout the 16-day observation period.

A flock of 800 white leghorn laying hens was sprayed with 0.25% Ciodrin for the control of the chicken body louse (Eomenacanthus stramineus). Spraying was accomplished by dividing the flock into three groups and spraying each group as a unit. Ten gallons, or about 47 ml./bird, was used for the flock and an attempt was made to treat both the tops and bottoms of the birds; however, coverage was considered only fair. Forty birds, chosen at random, were examined before and at intervals after treatment. Prior to spraying, all were heavily infested. Two days after spraying, 11 of 40 birds examined had lice, and only 2 birds had counts above 10 lice per bird. From 2 weeks to 4 weeks after treatment, from 5 to 13 of the 40-bird samples had lice and all counts were below 10 lice per bird. At 5 weeks after treatment, 11 of the 40 birds examined had lice and the population of lice per bird was increasing; one bird had 50 lice.

Preliminary surveys with northern fowl mites, taken from live birds at Hansen's Leghorn Ranch in Corvallis, Oreg., indicated that approximately the same order of resistance to malathion existed as when the population at this ranch was first assayed in 1958. Some of the mites were used to infest birds at the laboratory. Mites from this laboratory colony were used in further tests, in which malathion alone was tested in comparison with malathion combined with each of three synergists in a 1:1 ratio. Approximate LD-50 and LD-90 values for the malathion alone were 0.0072% and 0.017%. For malathion plus ENT-25812, the values were 0.00185% and 0.0029%, respectively; for malathion plus ENT-25989, the values were 0.00066% and 0.0012%, respectively; for malathion plus ENT-25988, the values were 0.00044% and 0.0005%. The malathion-ENT-25812 combination was 4 and 6 times more toxic than the malathion alone; the combination with ENT-25989 was 11 and 14 times more toxic than malathion alone; the combination with ENT-25988 was 16 and 34 times more toxic than the malathion alone. As these synergists are believed to be specific in restoring susceptibility to malathion in some malathion-resistant insects (flies

and mosquitoes), the studies suggest some resistance is developing to malathion in a population of the northern fowl mite.

Tests were conducted in Mississippi with Dowco-175 and Ciodrin for control of the northern fowl mite (Ornithonyssus sylviarum). Both chemicals were used as a spray (40 ml./bird) at concentrations of 0.05%, 0.1% and 0.25% and as a dip at these concentrations and at 0.025%. When used as a dip, Dowco-175 was effective at all concentrations except the lowest (0.025%), which failed after 20 days. As a spray, Dowco-175 at 0.05% was ineffective. Mites were present 28 days after treatment at 0.1%, but not at 0.25%. As a 0.25% dip, Ciodrin was completely effective after 28 days. However, as a dip at other concentrations, this material failed between 20 and 23 days after treatment. The lowest spray concentration, 0.05%, was ineffective; the treatment at 0.1% failed after 20 days and the one at 0.25% failed after 23 days.

4. Fleas. In Texas, white leghorn hens having sticktight fleas (Echidnophaga gallinacea) were sprayed or dipped in Dowco-175 or Ciodrin. Each bird was treated individually. In the Dowco-175 tests, fleas were eliminated at 0.1% and above, but not at 0.05%. In the Ciodrin tests, fleas were eliminated by both sprays and full dips. Tail dipping reduced the flea population by about 65% at 10 days, but the number of fleas was increasing again by the 20th day. When sprays were used, about 40 ml. was applied per bird. A flock of 26 white leghorn hens, heavily infested with sticktight fleas was dipped in 0.25% suspension (prepared from a wettable powder) of ENT-17470. The henhouse was sprayed with 1/2 gallon of the suspension and the nest boxes and nest material replaced. No control of the fleas was evident 4 days after dipping the birds and the entire flock was redipped in 0.25% ENT-17470 in emulsion form, again with no control. One week later, the flock was dipped from head to the base of the wings in 0.25% emulsion of ENT-25780. An estimated 95% mortality of the fleas was observed 2 and 5 days after this dipping, but by the 12th day, the flea population had increased to 10-25 per bird. The birds were redipped in 0.25% ENT-25780, 20 days after the first treatment with this material. Seven days after this second treatment, the birds were clean of fleas, but at 14 days, 1 flea was found and at 21 days, 6 fleas were found on the flock.

C. Insecticide Residue Determinations

1. Toxicity Studies. Initial tests with Dowco-175 in Texas to determine maximum levels chickens could tolerate were conducted with this chemical in sprays (40 ml./bird) and dips, at concentrations ranging from 0.05% to 1.0%. Birds dipped in 1.0% showed evidence of toxicity but recovered following atropine therapy supplied by a cooperating Animal Disease and Parasite Research Division veterinarian.

D. Insect Sterility, Attractants, and Other New Approaches to Control

1. House Flies. Research on the development of sterilization for the control or eradication of house flies has been continued in Florida and Oregon. In

Florida, investigations involved the screening of candidate chemosterilants, secondary laboratory tests with promising compounds, experiments related to actual field conditions, experiments with natural populations, and fundamental studies on effects of chemosterilants on house fly physiology and cytology. Eight hundred and eighty six new chemicals were screened for sterilant activity. Of these some produced toxic effects; however, 90 caused complete or partial sterility in the treated house flies. Many promising compounds have been further evaluated for their effects on individual sexes, their maximum tolerated as well as minimum effective dosage, and other methods of administering the compounds to house flies. Seven compounds were highly effective in sterilizing both sexes of house flies, but two of these were also toxic at the dosages tested. Metepa and tepa as residual deposits on glass sterilized house flies at dosages from 25 to 250 mg. per sq. ft., but 5-fluoroorotic acid was ineffective as a residual deposit at similar dosages. Apholate in the adult food sterilized house flies regardless of their age and the flies did not regain fertility. Motile sperm were present in the testes of chemosterilized males and transferred during copulation to the spermathecae of females throughout the life span of the male.

Tests were initiated to determine whether a dosage of chemosterilant too low to prevent hatching or adult emergence might by the accumulation of small genetic injuries eventually reduce or eliminate reproduction. With apholate, one colony showed reduction of oviposition in the 4th, 5th, 6th, and 7th generations and no individuals reached the pupal stage in the 7th generation. With metepa, the 5th through 9th generation of another colony showed reduced oviposition and the 10th generation failed to oviposit.

Preliminary tests with apholate, tepa, and metepa, and house flies indicated that the probit of the percent sterility with house flies can be related to the log of the concentration.

Metepa and apholate shortened the life span of adult house flies considerably, but 90% or more of the male population survived the first 10 days, or that period of time in which mating activity is the greatest. Survival during the first 10 days was essentially the same in treated and non-treated house flies. In other experiments house fly pupae were dipped in solutions of tepa, apholate, or metepa. This treatment was toxic to most individuals, but a high degree of sterility occurred in individuals surviving the treatment. Tests have been conducted with various formulations and types of baits for administering chemosterilants to house flies. Effective formulations of both dry baits and paint-on liquid baits have been developed.

Studies conducted in Oregon with an olfactometer and with simulated treated fly models (pseudo flies) demonstrated the presence in female house flies of a volatile chemical or chemicals which can influence the behavior of male flies. The behavior modification elicited was in the nature of attraction to a source of the pheromone, or an excitation of mating behavior patterns. The

material, which is benzene soluble and relatively stable, was shown to be sex related, and appeared to be specific to the house fly since extracts of neither the face fly or stable fly affected the behavior of male house flies.

In Oregon tests were conducted to determine sterilizing effects of tepa and metepa on the little house fly (Fannia canicularis). All flies feeding on bait containing 0.1% tepa for 4 days and 0.5% tepa for 2 days succumbed in 3 and 5 days, respectively, and none oviposited before death. Flies feeding on 0.05% tepa for 3 days survived normally but did not oviposit. Metepa at 0.5% was highly toxic to flies. Those feeding on baits containing 0.05 and 0.1% metepa survived and laid a few eggs but none hatched. Longevity of flies exposed for 2 hours on 100 mg. per sq. ft. residues of tepa was greatly reduced and all flies were fully sterilized. Flies exposed on residues of 1 and 10 mg./sq. ft. laid a few eggs but none hatched. These tests indicate that the little house fly is much more easily sterilized than the house fly. Over 50 inorganic and organic materials were tested as attractants for the little house fly. None showed significant attractancy.

In cooperation with the Agricultural Engineering and Animal Husbandry Research Divisions, studies were continued in new facilities at Beltsville, Md., to develop physical and mechanical methods of controlling house flies and other flies. Colonies of flies were established for the conducting of laboratory and outdoor cage studies. Studies to date have been concerned primarily with the attractiveness of various flies to different kinds and intensities of light. Black light untraviolet radiation was attractive to both house flies and face flies during twilight periods. The use of fluorescent panels behind light sources appeared to increase the attractiveness.

2. Mosquitoes. In Oregon tepa and ENT-50450 were tested as sterilants against Culex tarsalis adults by spraying in wind tunnel tests and against larvae by exposure in water. Tepa sterilized males and females at a spray concentration of 6%; ENT-50450 sterilized only males at this concentration. Lower concentrations of either compound were not effective. Against larvae, both compounds were toxic at higher test concentrations and ineffective and partial toxicants at lower concentrations.

In Oregon studies have shown that grass infusion and log pond waters are attractive to ovipositing female Culex pipiens quinquefasciatus. Studies have shown that distilled water treated with methane or furfural are more attractive to ovipositing females than distilled water, but less attractive than log pond water.

PUBLICATIONS REPORTING RESULTS OF USDA AND COOPERATIVE RESEARCH

Basic Biology, Physiology and Nutrition

Bigley, W. S. and F. W. Plapp. 1962. Metabolism of malathion and malaoxon by the mosquito Culex tarsalis Coq. Ins. Phys., 8:545-57.

Eddy, G. W., A. R. Roth, and F. W. Plapp. 1962. Studies on the flight habits of some marked insects. Jour. Econ. Ent. 55: 603-7.

Riley, Jack and R. A. Hoffman. 1963. Observations on the meteorological--mosquito population relationship at Stoneville, Mississippi, 1959-60. Mosq. News 23: 36-40.

Woodard, D. B., D. E. Weidhaas, and H. R. Ford. 1962. A method to study mating activity of Anopheles quadrimaculatus Say under field conditions. Fla. Anti-Mosq. Assoc., 33: 24.

Insecticidal and Sanitation Control

Chapman, H. C. and L. L. Lewallen. 1963. Field tests with insecticides against mountain Aedes larvae in California. Mosq. News 23: 41-4.

Gahan, J. B., G. C. LaBrecque, and H. G. Wilson. 1962. Relative toxicity of selected compounds as residual sprays against adults of Anopheles quadrimaculatus Say. Mosq. News 22: 333-6.

McDuffie, W. C., J. B. Gahan, A. N. Davis, and G. C. LaBrecque. 1962. Highlights of recent research on mosquito control by the Entomology Research Division, USDA. Proc. 49th Ann. N. J. Mosq. Exterm. Assoc. Meeting, pp. 16-27.

Insect Sterility, Attractants, and Other New Approaches to Control

Dame, David A. and Claude H. Schmidt. 1962. The importance of competitiveness of radiosterilized males in mosquito control programs. Proc. 49th Ann. Meet. N. J. Mosq. Exterm. Assoc., pp. 165-8.

LaBrecque, G. C., D. W. Meifert, and R. L. Fye. 1963. A field study on the control of house flies with chemosterilant techniques. Jour. Econ. Ent. 56: 150-2.

LaBrecque, G. C., D. W. Meifert, and H. K. Gouck. 1963. Effectiveness of three propylenimine derivatives as house fly chemosterilants. Fla. Ent. 46: 7-10.

LaBrecque, G. C., Carroll N. Smith and D. W. Meifert. 1962. A field experiment in the control of house flies with chemosterilant baits. Jour. Econ. Ent. 55: 449-50.

LaChance, L. E. and E. F. Knipling. 1962. Control of insect populations through genetic manipulations. Ann. Ent. Soc. of America 55: 515-20.

Plapp, F. W., Jr., W. S. Bigley, G. A. Chapman, and G. W. Eddy. 1962. Metabolism of methaphoxide in mosquitoes, house flies, and mice. Jour Econ. Ent. 55: 607-13.

Weidhaas, Donald E. 1962. Chemical sterilization of mosquitoes. Nature, 195(4843), pp: 786-7.

Weidhaas, Donald E. and Claude H. Schmidt. 1963. Mating ability of male mosquitoes, Aedes aegypti (L.), sterilized chemically or by gamma radiation. Mosq. News 23: 32-4.

Weidhaas, Donald E., C. H. Schmidt, and E. L. Seabrook. 1962. Field studies on the release of sterile males for the control of Anopheles quadrimaculatus. Mosq. News 22: 283-91.

AREA NO. 17. INSECTS AFFECTING MAN, HOUSEHOLDS, AND INDUSTRIAL ESTABLISHMENTS

Problem. Insects, ticks, and mites are responsible for widespread human misery and certain insects cause heavy losses of food and materials in households and industrial establishments. Many of the same or closely related insects which affect man are also important pests of livestock, thus research on insects in relation to man and to livestock is mutually advantageous. Certain arthropods are vectors of major diseases which annually cause the deaths of millions of humans. Mosquitoes, for example, transmit malaria, dengue, encephalitis, yellow fever, and filariasis. Some of these diseases, as well as other arthropod-borne diseases, occur and are potentially serious problems in the United States but most of them are of more concern in other parts of the world, where troops and civilian personnel are maintained. The military agencies have for many years depended on the research competence in agriculture for answers to their military insect and insect-borne disease problems. Attacks by insects, ticks, and mites frequently prevent farm and forest work, reduce or destroy the value of recreation areas, and even make certain areas uninhabitable. Property values are often depressed and development prevented by hordes of annoying pests. Mosquitoes, bed bugs, and fleas are frequently serious annoyances in homes. Other household insects are of economic importance in homes and industrial establishments because they damage foods, fabrics, and other materials, causing losses of millions of dollars annually. There is a great need for safe, economical insecticides and satisfactory methods for their application that could be used quickly and effectively to control local infestations or outbreaks of pests that annoy man in the field or at home, especially where there are threats of disease epidemics. Improved means for controlling mosquitoes, sand flies, gnats, the imported fire ant, and similar pests, should receive particular attention. More efficient repellents are needed to protect humans, particularly when other means of control cannot be employed. Special efforts should be made to develop systemic materials which, when taken orally, would repel or prevent insects from biting. Sanitation, habitat management, and other non-insecticidal methods of control should be reappraised, and biological control, especially with insect pathogens, needs to be fully explored. New approaches to control, including chemosterilants, antimetabolites, attractants, and radiation require intensive investigation. Studies should be undertaken on the biology, ecology, physiology, and genetics of many important pests affecting man and the household in order to find weak points in their life cycles which might be utilized to improve control efficiency.

The Department has a continuing program involving applied and basic research on insects affecting man, households,and industrial establishments, including mosquitoes, house flies, human lice, bed bugs, gnats, fleas, ticks, mites and other pests of man, and on cockroaches, ants, and several other pests of importance in households and industrial establishments. Research is directed toward the development of more effective insecticides and repellents and involves primary screening of chemicals and field evaluation of promising

materials. Investigations are conducted on the nature of insect resistance to insecticides, on the mode of action of insecticides, on the effects of radiation and chemosterilants, on attractants, on factors affecting attraction of biting insects to humans, and on the factors affecting the effectiveness of repellents. Attention is also given to the development of sanitation and management procedures and to biological control methods for mosquitoes, house flies, cockroaches, and several other pests. Studies are conducted in cooperation with the Agricultural Engineering Research and Animal Husbandry Research Divisions to develop physical and mechanical methods of insect control and to evaluate various kinds of traps and devices for estimating and controlling natural populations of flies, mosquitoes, and other pests. Studies are also conducted in cooperation with the Soil and Water Conservation Research Division of ARS and the Bureau of Vector Control, California State Department of Health, on soil and water management procedures to prevent mosquito production in western irrigated areas and in log ponds. The major portion of the program is now conducted at Gainesville, Fla., following a transfer in June 1963 of the investigations from Orlando, Fla., where the research herein reported was carried out. The remainder of the work is done at Corvallis, Oreg., Fresno, Calif., and Beltsville, Md. Close cooperation and evaluation of research data and needs are maintained with the Defense Department through the Armed Forces Pest Control Board concerning studies on insects important to military personnel. Research funds supporting 1 1/2 professional man-years have been transferred from the Defense Department to support research on chemosterilization of house flies. Cooperation is maintained with the World Health Organization on studies developing new insecticides and other methods of control on insects affecting man. The World Health Organization provides financial support (1/2 professional man-year) for studies at Gainesville, Fla., on the development of residual insecticides for mosquito control.

The Federal scientific effort devoted to research in this area totals 20.1 professional man-years. Of this number 3.1 is devoted to basic biology, physiology, and nutrition; 9.1 to insecticidal and sanitation control; 0.7 to biological control; 5.7 to insect sterility, attractants and other new approaches to control; 0.5 to the evaluation of equipment for insect detection and control; and 1.0 to program leadership.

Additional research (2 professional man-years) has been initiated under a grant of P.L. 480 funds (S9-ENT-3) to the Facultad de Agronomia, Universidad de la Republica, Uruguay, on "Investigations on the biology and biological control of the fire ant, Solenopsis saevissima richteri, in Uruguay."

REPORT OF PROGRESS FOR USDA AND COOPERATIVE PROGRAMS

A. Basic Biology, Physiology, and Nutrition

1. Mosquitoes. At Orlando, Fla., studies on the biology and behavior of mosquitoes were continued to obtain basic data applicable to the development of new approaches to control, particularly the sterility method. A two-year

study on Anopheles quadrimaculatus biology under field conditions has been concluded. Data have been obtained on population levels, climatic conditions, mating activity, dispersion, and physiological age and insemination rates of laboratory as well as wild strains. Techniques utilizing sterilization by radiation or chemicals along with tagging with radioisotopes have been developed and with these it has been possible to show that colonized A. quadrimaculatus mosquitoes have behavioral, mating, dispersion, and biting traits which differ considerably from the same traits in wild mosquitoes of the same species. The ability to sterilize mosquitoes with chemicals or radiation has offered an excellent tool for basic studies of mosquito biology and mating behavior under either laboratory or field conditions.

Laboratory studies with the salt-marsh mosquito have defined conditions essential for a high degree of mating activity and egg viability, which will permit experiments on chemosterilization of this mosquito. A series of laboratory tests with adult A. quadrimaculatus did not show the presence of a chemical attraction of either sex for the other.

Surveys and studies on the occurrence and biology of Anopheles albimanus were conducted on San Salvador, one of the Atlantic Missle Range Islands. Preliminary data have been accumulated on population levels, breeding sites, insemination rates, and physiological age of wild females.

At Corvallis, Oreg., and Fresno, Calif., studies were continued on the biology and ecology of important mosquito species. In laboratory tests the majority of Culex tarsalis females preferred to feed at the higher of two feeding locations and preferred mice rather than chickens for their source of blood.

A study was conducted on the daily activity of several species of mosquitoes--Anopheles freeborni, Culex peus, Culiseta inornata, Culex incidens, and Culex tarsalis. All species behaved similarly. Both males and females began to move out of daytime resting shelters a few minutes before or after sunset. Only a few males and females (0.3 to 2.8%) remained in the shelters during the night. Adults did not begin to return to the shelters until sunrise or a few minutes later, and all did not return until sometime after 8:00 a.m. Exodus from and return to shelters appeared to be regulated by light intensity, but some other factors such as preconditioning to light cycles, temperature, and humidity may have had some effect since artificial lights did not prevent mosquitoes from leaving shelters eventually, but did change their time and rate of leaving. Use of special crosses of resistant and susceptible strains of Culex tarsalis showed that some of the females mate more than one time and utilize sperm from more than one mating.

Taxonomic studies in California have defined suitable characters for separating dark-winged forms of Aedes dorsalis from A. melanimon and have shown that these two forms coexist in only one area (Solano County). Surveys at Borrego Springs (San Diego County) were made to find isolated populations of mosquitoes for future studies on control through sterilization.

Surveys were continued on biting arthropods of the Humboldt River Basin in Nevada. During April, May, and June, water was plentiful and high populations of Aedes melanimon, A. dorsalis, A. vexans, Culex tarsalis, and Culiseta inornata were found. Mosquito larvae were infected with a microsporidian, Thelohania sp. and a bacterium,both of which are under study as biological control agents. In late July, no floodwater Aedes breeding was observed, but moderate breeding of Culex tarsalis and Culiseta inornata was observed. Studies of mosquito larval populations in typical breeding areas in dairy drains and culverts in California during April indicated the presence of Culiseta inornata, Culiseta incidens, Culex tarsalis, Culex peus, Culex apicalis, and Anopheles franciscanus, but no breeding of Culex quinquefasciatus was found.

Using C-14 labeled TDE, slight but consistent differences in its rate of degradation have been noted in susceptible and resistant strains of Culex tarsalis indicating that the main source of resistance to this chemical is increased degradiation of TDE to FW-152 and water soluble metabolites through an oxidative rather than a dehydrochlorination mechanism. Further tests with DDT and TDE have shown that analogs of DDT which block metabolism via the oxidative pathway overcome resistance to DDT and tend to confirm the hypothesis that resistance to DDT and TDE in tarsalis is a more rapid oxidative metabolism of the insecticide.

2. House Fly. At Orlando, Fla., research on control or eradication through the use of sterilization by radiation or chemicals has shown that considerable gaps exist in knowledge of the biology and mating behavior of house flies. Both males and females must undergo a sexual maturation time of at least 16 hours with males and a minimum of 24 hours with females. Once the mating drive has started, males will attempt to "strike" or mate with both males and females and certain inanimate objects, although they "strike" more readily and frequently with females. This fact along with experiments in a large cage-type chemotactometer suggested the presence of some type of a female sex attractant of a low order. Imperfect sex recognition in the male combined with a low order sex attractant in the female would account for the fact that males attempt to mate with either sex and result in the higher ratio of male-to-female "strikes." Males or females which had their wings removed were able to mate with individuals of the opposite sex with wings. Amputation of more than one pair of legs from the male prevented mating, while amputation of only one pair of legs impeded but did not prevent mating.

Tests were run to determine the actual time house flies remain in copula after initial seizure. Of 61 mating pairs that were trapped and observed, the shortest mating period was 44 minutes and the longest, 96 minutes. The average was 60 minutes. After mating periods of only 1 and 2 minutes, no sperm were found in females, but after 3 to 5 minutes some spermathecae contained a few sperm and some were completely filled. For periods of 10 to 76 minutes spermathecae were filled to capacity with sperm with only one exception.

Observations on the natural sterility of 86 female house flies reared in the laboratory gave an average of 94.4 eggs per female of which 89% hatched. The number of eggs laid varied from 12 to 186 per female. Some flies fed within an hour after emergence, others did not feed until later, and some did not feed until they were 16 to 18 hours old.

At Corvallis, Oreg., studies were continued on the physiology of resistant and susceptible house flies. Isolan-resistant house flies have decreased ali-esterase activity and increased Isolan-detoxifying enzyme. Techniques using high-speed centrifugation have been developed which concentrate enzymes important in metabolizing or conferring resistance to organophosphorus compounds. Experiments with malathion-resistant house flies indicate that the nature of the alkyl groups attached directly to the phosphorus atom is the major factor in resistance. Thus, the primary cause of resistance is probably associated with the rate of recovery of the phosphorylated detoxifying enzyme after poisoning, rather than to an increased ability to cleave the toxic molecule per se.

3. Eye Gnats. Colonization of Hippelates pusio over several years has not induced behavior or mating changes in the colonized individuals. In tests to determine the best combination of CSMA and vermiculite for rearing pusio, a ratio of 1:1 was found to be the most favorable.

An extensive study of the male reproductive system of pusio has been completed. Descriptions of the structure and function of the parts of the male reproductive system--vas differens, accessory glands, ejaculatory duct--have been made. The development of testes and sperm has been followed as males were aged. Tests with males of various age showed that mating did not have any gross observable effect onthe testes. However, the density of spermatozoa in the ejaculatory duct increased with age.

Research with female pusio has shown that follicular development within the ovaries of both mated and unmated females follows closely the five stages described for mosquitoes. When the follicles of mated females were in stages IV and V the spermatozoa were always in the bursa. During other follicular stages, the spermatozoa were always in the spermathecal ducts.

Unmated pusio females were capable of laying infertile eggs; however, they required a longer preoviposition period and produced slightly fewer eggs than mated females. Fertile eggs could be stored for 14 days with no indications that storage reduced their viability. High humidities (70% R.H. and over) were more favorable for survival of and egg production in adult gnats.

4. Ants. Information on the distribution, biology, and economic importance of the fire ant, Solenopsis saevissima richteri, in Uruguay has been provided in a report from investigators working in that country under P.L. 480 project (S9-ENT 3). The abundance of ants was greatest in cultivated areas. Damage to mowing equipment, rice field levees, and cyclamen bulbs and stinging of personnel working in areas where the ants occur, were reported. This ant was

associated with 22 species of plants and many insects other than ants were found in the mounds. Possible predation or parasitism of this ant by a mite, a jumping spider, a chalcid wasp, and another species of ant occurred.

B. Insecticidal and Sanitation Control

1. Mosquitoes. Studies were continued at Orlando, Fla., to find new and effective insecticides for the control of mosquitoes. In screening tests with Anopheles quadrimaculatus larvae, 28 of 81 compounds tested were rated Class IV in effectiveness. Four of these compounds--American Cyanamid CL-18133, Stauffer B-10046, Stauffer 8-10094, and Bayer 47940--were highly effective, killing 100% of the larvae at concentrations of 0.05 p.p.m. to 0.01 p.p.m. One hundred and nine plant extracts were also screened for toxicity, but none of these were toxic at low concentrations. Of 83 compounds tested against female Aedes taeniorhynchus in wind tunnel tests, 7 were as effective as the standard, malathion. Dimethrin in granular formulations was effective in laboratory and field tests as a larvicide against Anopheles quadrimaculatus, Aedes aegypti, Aedes taeniorhynchus and Culex quinquefasciatus mosquitoes. A mixture of DDT and anti-resistant compound was no more effective than DDT alone against C. quinquefasciatus larvae.

Field tests were conducted in the Panama Canal Zone to determine the effectiveness of some of the more recently developed insecticides against mosquitoes and to establish a program whereby military entomologists in the field can assist in conducting field evaluations of promising insecticides. Selected insecticides were tested against natural populations of mosquitoes by aerial application, against caged insects by dispersal with the Army cold fogger, and as residual insecticides in horse-baited traps. In tests with caged insects and ground fogging equipment, naled and malathion gave effective control of adult mosquitoes, but DDT did not. In aerial applications, none of these insecticides gave satisfactory control because of dense jungle canopy and migration of mosquitoes into the test plots; however, DDT gave as good control as naled and malathion. Evaluation of the residual deposits has not been completed.

In Florida, extensive screening tests to evaluate new compounds as residual treatments for control of malaria mosquitoes were continued. Among the most effective materials or mixtures tested were Bayer 39007, Bayer 39007 plus Monsanto CP-16226, Bayer 41831, Bayer 37344, Bayer 25141, Hercules 7522H, Hercules 7522H plus piperonyl butoxide, Hercules 7522H plus Monsanto CP-16226, Hercules 7527G, Hercules AC-5227, Hercules AC-5227 plus piperonyl butoxide, Hercules 9699, Monsanto CP-16226, Monsanto CP-40273, Stauffer N-2230, Stauffer 2310, Sevin plus synergist, Upjohn U-12927, and Zectran, all of which were at least 70% effective at 100 mg./sq. ft. through 48 weeks of aging.

At Fresno, Calif., and Corvallis, Oreg., studies on the development of insecticides for mosquito control were continued. In the laboratory 7 of 9

chemicals tested against Culex tarsalis adults in wind tunnel tests were more effective than malathion. In tests with C. tarsalis larvae, eight compounds were highly effective, killing 100% of the larvae at concentrations of 0.05 p.p.m. to 0.0025 p.p.m. In small plot field tests against larvae of snow pool mosquitoes, Bayer 29493 and parathion were the most effective of the compounds tested and Hercules AC-5727, malathion, carbophenothion, and naled gave good control (92% or better). In treatment of log ponds for mosquito control, Bayer 29493 in granular formulation at 0.1 lb./acre gave excellent control and showed some residual effectiveness.

2. House Fly. Research was continued at Orlando, Fla., to develop more effective insecticides and other methods and materials for the control of house flies. Twenty-three new compounds were tested as space sprays in a wind tunnel against the regular susceptible colony and the Cradson (multi-resistant) colony. Fourteen of the compounds were more effective against both the susceptible and resistant colonies than the standard, malathion. New insecticides were also evaluated as residual treatments against female house flies from the regular or Cradson colonies. The criterion of effectiveness was the number of weeks of aging during which the residues remained effective in killing house fly females. Against susceptible house flies Hercules AC-5727 alone and Bayer 39007 alone gave kills of 90% for one week, but in combination with Monsanto CP-16226, their effective periods were extended to 8 and 12 weeks, respectively. The effectiveness of Hercules 7522H was also extended from 1 to 12 weeks with the addition of Monsanto CP-16226. Against flies of the Cradson colony, all of these formulations failed before the fourth week. Against the regular colony Bayer 29952 and Bayer 30237 were effective for 64 and 56 weeks. Stauffer N-2230 and Stauffer N-2404 were 100% effective throughout 48 weeks of aging against the susceptible colony, but they were ineffective against the Cradson colony. General Chemical GC-3583 was still 100% effective after 96 weeks against the regular colony and Monsanto CP-40294 was effective for 24 weeks against the Cradson colony. Against house flies from the susceptible colony, General Chemical GC-4072 was 100% effective for 96 weeks as an acetone solution and Stauffer N-2310, Bayer 39197 and Monsanto CP-40273 for 48+ weeks. As wettable powder, DDT was more than 90% effective for 48 weeks, Bayer 25141 for 48 weeks, Bayer 34098 for 40 weeks, Hooker HRS-1422 for 32 weeks, and Bayer 32651 for 28 weeks.

Residual tests (deposits of 100 mg./sq.ft.) were conducted with emulsions of Diazinon, Baytex, and dimethoate against house flies in Florida dairy barns. The diazinon treatment failed to give satisfactory control as early as the first day after treatment. Baytex gave 97% control for 5 days and from 79% to 88% control through 14 days. Dimethoate gave controls ranging from 80% to 96% for 6 weeks, when the test was discontinued.

Windtunnel tests have been conducted in Florida to compare the susceptibility of house flies from the regular colony and eight experimental colonies to various insecticides. House flies of the experimental colonies showed high levels of resistance to one or more of the following insecticides: DDT,

malathion, parathion, Co-ral, and Sevin. A proposed experiment for the eradication of house flies on one of the Atlantic Missile Range Islands will employ the use of malathion baits with the simultaneous release of malathion-resistant, chemosterilized flies to reduce the fertility of that segment of the population not killed by the bait.

At Corvallis, Oreg., extensive studies were continued on the development of synergists that have overcome resistance to organophosphorus insecticides in both house flies and mosquitoes. Of some new types of compounds screened, results indicate that diisopropyl or dibutyl substituents would be most satisfactory. Selection of house flies with combinations of malathion and synergists is being carried out to determine if resistance to the combinations can be developed.

3. Cockroaches. Research was continued at Orlando, Fla., to develop new insecticides for controlling cockroaches. Of 50 new compounds screened as contact sprays, 16 were promising and gave 100% mortality in 24 hours at a concentration of 0.5%. Of 10 new compounds screened for residual effectiveness against normal German cockroaches, 8 caused 100% mortality in 24 hours after a 30-minute exposure to fresh deposits of 100 mg. per square foot. Two materials--Stauffer N-2230 and Stauffer N-2404--tested as residues were superior to the chlordane standard and caused 100% and 85% mortality, respectively, of German cockroaches after 8 weeks of aging.

Laboratory tests were conducted with Kepone and mirex baits with American and resistant and susceptible German cockroaches. Though effective in killing cockroaches, the two baits were much slower acting than the standard Dipterex bait. However, in a field test over a period of weeks, the Kepone bait gave a high degree of control of American and German cockroaches. Preliminary studies with DDVP as a vapor toxicant against cockroaches indicate that it would require an exposure period of greater than 24 hours to produce 100% kill with a concentration of 1.0 microgram of DDVP per liter of air.

The repellent action of 5 insecticides commonly used as insecticides for cockroach control was evaluated. Chlordane, diazinon, ronnel, and malathion did not function as repellents to the degree that they failed to be effective toxicants. The combination of pyrethrins and piperonyl butoxide repelled cockroaches.

4. Body Lice. Research was continued at Orlando, Fla., to develop more effective insecticides for the control of body lice. Of 299 compounds screened, 84 were rated Class IV and IVA in toxicity and 59 were Class IV and IVA in speed of knockdown. Of materials tested as synergists for Sevin against body lice, none were more effective than the standard sulfoxide, but 2 of them extended the residual effectiveness of Sevin slightly more than sulfoxide did. In patch tests, four compounds--Bayer 39007 and Hercules 9699, Hercules 9326 and California Spray RE-5305-3--were more effective than the standards (DDT and lindane).

Extensive sleeve tests on human subjects were conducted with combinations of Sevin and sulfoxide to develop an effective louse powder. A powder containing 4% of Sevin plus 2% of sulfoxide was 100% effective for 20-21 days after treatment and 95%-98% effective 27-28 days after treatment. A powder containing 2% of Sevin plus 2% of Sulfoxide was 100% effective for 17 days after treatment and 81% to 91% effective 27 days after treatment. The standard malathion powder was 100% effective for 24 days and 97% to 98% effective for 27-28 days after treatment. Cholinesterase levels of the human subjects who wore the treated sleeves remained about the same as before treatment.

Studies were continued on the development of resistant strains of body lice and their susceptibility to various insecticides. Strains that are slightly to highly resistant to one or more of the following compounds--DDT, lindane, Sevin, malathion, and activated pyrethrins--have been developed and studied. No evidence of the development of resistance to one compound with resultant development of susceptibility to another compound has been found.

5. Mites, Ticks, and Fleas. Screening tests were conducted in Florida to find new insecticides effective against fleas. Of 33 compounds tested against oriental rat fleas, 15 were at least as effective as the standard, malathion, in that they caused complete mortality of fleas exposed to residues which had been aged for 12 weeks.

To evaluate compounds against oriental rat fleas under conditions similar to natural situations, a procedure has been developed for the evaluation of the better compounds found in screening tests. The technique involves the application of insecticide dusts to soil and the subsequent exposure of fleas to the treatment. In this type of test Bayer 37344 was the most effective of 9 compounds tested. It caused 100% mortality of fleas exposed to concentrations in dust as low as 0.1%. DDT, Bayer 29493, Hercules AC-5727, lindane, diazinon, and malathion caused 100% mortality at concentrations as low as 1%. At a concentration of 5%, Bayer 39007 and Hercules 7522C produced mortalities of 100%, but at 1% the mortalities were 97% to 98%

6. Bed Bugs. Research at Orlando, Fla., to discover and develop new insecticides for the control of bed bugs was continued. Forty-one candidate compounds were screened against bed bugs by exposing them to residues of the insecticides on filter paper (50 mg. per square foot). Of these compounds, 12--Bayer 29493, Bayer 30237, Bayer 30554, Bayer 29492, Bayer 41831, carbophenothion, Ciodrin, Dicapthon, EPN, Hercules AC-5727, Methyl Trithion, and Stauffer N-2404--were equal to or better than the standard, malathion, since they caused 95% to 100% mortality of bed bugs when bed bugs were exposed for 24 hours to residues that had aged 8 to 12 weeks.

Research has been conducted to develop standardized techniques for evaluating the presence and degree of resistance to insecticides in bed bugs. Studies with malathion and Bayer 29493 have determined the concentrations and exposure times to be used with these two insecticides to allow detection of susceptible and resistant strains of bed bugs.

7. Ants. Mirex bait, a formulation containing 0.25% mirex and peanut butter, was evaluated for its effectiveness in controlling a natural infestation of Pharaoh ants in insectary rooms of the Orlando, Fla., laboratory. The bait, placed in feeding stations constructed of small plastic lids covered with screen that permitted entry of the ants, eliminated the ants from the insectary rooms.

C. Biological Control

1. Mosquitoes. At Fresno, Calif., in cooperation with the Bureau of Vector Control, California State Department of Health, studies were initiated on biological control agents for mosquitoes. Studies have been initiated on the host, parasite, and host-parasite relationship of mosquitoes and various species of microsporidia of the genus, Thelohania.

D. Insect Sterility, Attractants, and Other New Approaches to Control

1. Mosquitoes. Extensive studies on the chemosterilization of mosquitoes were continued at Orlando, Fla. In laboratory tests, none of 84 compounds evaluated as larval sterilants against Aedes aegypti were as effective as apholate which consistently gave 94-100% sterility, but 5 compounds reduced egg hatch to 20% or less. Various combinations of apholate and tepa were no more effective as larval sterilants than either chemical alone. Granular formulations of apholate (pyrophyllite granules, corncob grits, buffered vermiculite granules) were slightly less effective than solutions of apholate in sterilizing A. aegypti larvae. When tested in the presence of soil, the sterilant activity of granular formulations and solutions of apholate was severely diminished. Formulations of apholate on pyrophyllite dust (which floats on water surface) gave consistently higher sterility in A. aegypti larvae than granular formulations under various test conditions including the presence of soil. At sterilizing concentrations tepa was more toxic to A.aegypti and Anopheles quadrimaculatus larvae than apholate. All instars of A. aegypti larvae were affected by treatment with tepa, but those exposed from the third instar to pupation had the highest degree of sterility. Under laboratory conditions, tepa sterilized A. aegytpi larvae for at least 6 days. Under field conditions, tepa was only partially effective and had no sterilizing effect after 3 days.

Pupae of Anopheles quadrimaculatus exposed to tepa were killed or failed to emerge at high concentrations. At lower concentrations, some individuals survived, but were not sterilized. Males of Aedes aegypti sterilized as larvae by treatment with apholate were capable of competing with normal males in mating normal females. In these tests individual females mated with both sterilized and normal males. With A. quadrimaculatus residual deposits as low as 0.34 mg./sq. ft. of glass surface completely sterilized adult females; males were not tested.

Females of Aedes aegypti sterilized completely by contact with residues of tepa did not transfer sterility to males during copulation. Males of

Anopheles quadrimaculatus treated with radiation at a level that did not induce complete sterility and by chemicals which caused complete or almost complete sterility competed equally with normal males for mating normal females. Both sexes of adult Aedes taeniorhynchus were rendered sterile by exposure to residual deposits of tepa and metepa.

In Oregon tepa and ENT 50450 were tested as sterilants against Culex tarsalis adults by spraying in wind tunnel tests, and against larvae by exposure in water. Tepa sterilized males and females at a spray concentration of 6%; ENT-50450 sterilized only males at this concentration. Lower concentrations of either compound were not effective. Against larvae, both compounds were toxic at higher test concentrations and ineffective and partial toxicants at lower concentrations.

In Florida 124 compounds were screened against mosquitoes as personal-use or clothing repellents and 236 for space repellency. None of those screened as space repellents showed a high degree of repellency. Five compounds screened as personal-use or clothing repellents showed sufficient promise for further testing. Storage tests were made with wrapped and unwrapped Army fatigue trousers and jackets. Garments treated with M-1960 and deet gave complete protection against Aedes aegypti after 6 months in storage. However, one week after removal from storage effectiveness was reduced. There was little difference in the effectiveness of deet as a skin repellent against A. aegypti when the deet was applied at similar concentrations as a pressurized foam, a zinc oxide ointment, or an ethanol solution. Deet stored in a tin can since November 1954 was as effective as a sample received in 1961 in tests with skin applications against A. aegypti

In Oregon studies have shown that grass infusion and log pond waters are attractive to ovipositing female Culex pipiens quinquefasciatus. Distilled water treated with methane or furfural was more attractive to ovipositing females than distilled water, but less attractive than log pond water.

2. House Flies. Research on the development of sterilization for the control or eradication of house flies has been continued. In Florida, investigations involved the screening of candidate chemosterilants, secondary laboratory tests with promising compounds, experiments related to actual field conditions, experiments with natural populations, and fundamental studies on effects of chemosterilants on house fly physiology and cytology. Eight hundred and eigthy-six new chemicals were screened for sterilant activity. Of these some produced toxic effects; however, 90 caused complete or partial sterility in the treated house flies. Many promising compounds have been further evaluated for their effects on individual sexes, their maximum tolerated as well as minimum effective dosage, and other methods of administering the compounds to house flies. Seven compounds were highly effective in sterilizing both sexes of house flies, but two of these were also toxic at the dosages tested. Metepa and tepa as residual deposits on glass sterilized house flies at dosages from 25 to 250 mg. per sq. ft., but 5-fluoro-orotic acid was ineffective as a residual deposit at similar dosages. Apholate

in the adult food sterilized house flies regardless of their age and the flies did not regain fertility. Motile sperm were present in the testes of chemosterilized males and transferred during copulation to the spermathecae of females throughout the life span of the male.

Tests were initiated to determine whether a dosage of a chemosterilant too low to prevent hatching or adult emergence might by the accumulation of small genetic injuries eventually reduce or eliminate reproduction. With apholate, one colony showed reduction of oviposition in the 4th, 5th, 6th, and 7th generations and no individual reached the pupal stage in the 7th generation. With metepa, the 5th through 9th generation of another colony showed reduced oviposition and the 10th generation failed to oviposit.

Preliminary tests with apholate, tepa, and metepa on house flies indicated that the probit of the percent sterility with house flies can be related to the log of the concentration. Metepa and apholate shortened the life span of adult house flies considerably, but 90% or more of the male population survived the first 10 days, or that period of time in which mating activity is the greatest. Survival during the first 10 days was essentially the same in treated and nontreated house flies. In other experiments house fly pupae were dipped in solutions of tepa, apholate, or metepa. This treatment was toxic to most individuals, but a high degree of sterility occurred in individuals surviving the treatment.

Tests have been conducted with various formulations and types of baits for administering chemosterilants to house flies. Effective formulations of both dry baits and paint-on liquid baits have been developed.

A method has been devised for studying chromosomes of house flies. Using this technique, the effect of chemosterilants on spermatogensis and oogenesis will be made.

Chemosterilant baits have been applied in privies on two islands (metepa on Grand Turk and apholate on Mayaguana) in the Atlantic Missle Range. On a third island (San Salvador), Dipterex bait was applied to provide a comparison between an insecticide and the chemosterilants. House fly breeding was limited to privies, almost exclusively, but the vast majority of feeding by house flies occurred in the kitchens. The baits were applied weekly at first, and then semiweekly. After several months the dry bait formulation was changed to a heavy sugar syrup which was sprayed in the privy pits. The metepa treatment on Grand Turk caused a high degree of sterility in eggs deposited by female house flies (usually 65% to 80% with dry baits and 80% to 86% with liquid baits). The total sterility may well have exceeded 95% since a high proportion of females did not lay eggs. The number of flies decreased gradually after treatment. There was a marked reduction in house fly populations, but complete eradication was not achieved after several months of treatment. Auxiliary methods of control have been initiated in an attempt to achieve complete eradication on this island. In other localities, where the principal feeding by house flies occurs outside houses in

places where baits might be applied, the bait alone might be sufficient. The dry bait treatments of apholate on Mayaguana were less effective than those of metepa on Grand Turk. This difference was attributed to the type of breeding places on the two islands. The abundance of flies was never high on San Salvador. Before treatment,grid counts ranged from 2 to 3 and after treatment they were less than 1.

In laboratory tests with the little house fly at Corvallis, Oreg., tepa and metepa administered either in the adult or as residual deposits sterilized surviving adult flies but were highly toxic at the concentrations or dosages used.

In tests in Oregon with an olfactometer and with simulated, treated fly models ("Pseudo flies"), the presence in female house flies of a volatile chemical or chemicals that can influence the behavior of male house flies was demonstrated. The behavior modification was in the nature of attraction to the source of the pheromone or an excitation of mating behavior patterns. The material, which was benzene soluble and relatively stable, was sex related and specific to the house fly.

3. Cockroaches. In Florida further tests were conducted with chemicals in the food or nymphal German cockroaches to determine their possibilities as sterilants. Apholate (2%) and metepa (1%) caused delayed development and were toxicants. Kepone (0.01%), aphomide (2%) and NSC-18016 (1%) caused delayed development and were partial toxicants. Methiotepa (0.25%) and methyl apholate (1%) caused delayed development in the nymphs, but methiotepa at 0.1% had no effect. Methiotepa and methyl apholate caused complete or reduced sterility in either sex of cockroaches and complete sterility when both sexes were treated.

4. Eye Gnats. Hippelates pusio can be sterilized with either apholate or tepa. However, in some cases mortality was high or the treatments were only partially effective. When chemosterilants were fed to adult gnats, tepa killed all or nearly all of the gnats at the 0.5% concentration. It also killed 58% of the gnats in the initial exposure at 0.1% but was not toxic thereafter or at lower dosages of 0.05% and 0.01%. Every concentration of tepa which was tested produced complete sterility when freshly prepared food was used. Apholate was much less toxic than tepa when fed to adult gnats. This compound sterilized all or nearly all of the gnats at concentrations of 0.5% and 0.1% in the adult food, caused some sterility at 0.05%, but was ineffective at 0.01%. In tests in which gnats were allowed to walk on residual deposits, tepa caused sterility at dosages as low as 3 mg./sq. ft. Apholate residues of 5 mg. or 10 mg./sq. ft. did not cause sterility in gnats. This work was conducted at Orlando, Fla.

5. Body Lice. At Orlando, Fla., tests were run to find chemicals that have systemic insecticidal or repellent action. In these tests candidate compounds were administered to rabbits orally by stomach tube or in their food, and body lice allowed to feed on the rabbits at various time intervals after

treatment. Of 63 compounds tested at various dosages (mg./kg.), 8 compounds caused complete mortality of lice without affecting the rabbits. The compounds and the lowest effective dosages were: Bayer 30468, 10 mg./kg.; Bayer 33904, 50 mg./kg.; Monsanto CP-19203, 10 mg./kg.; American Cyanamid CL-38064, 25 mg./kg.; Tandearil, 10 mg./kg.; Hercules AC-3273, 200 mg./kg.; American Cyanamid CL-26691, 10 mg./kg.; and American Cyanamid 18706, 100 mg./kg. Other compounds caused some or complete mortality of lice but affected or killed the rabbits at dosages which were effective for the lice. These compounds were Stauffer N-3422, American Cyanamid CL-38106, Monsanto CP-12432, heptachlor, and O, O-dimethyl 1-acetoxyethyl-2,2,2-trichlorophosphonate. Of 8 antibiotics tested for systemic activity against body lice, and butazolidin for systemic activity against the ear mite of rabbits, none were effective in killing lice or mites. A dose of 75 mg./kg. of Shell 52-RL-71 administered by stomach tube to rabbits caused 100% mortality of lice from 2-21 days after treatment.

6. Mites, Ticks, and Fleas. Research was continued in Florida on the develoment of repellents and other methods for protecting humans from mites, ticks, and fleas. One compound--Shell 52-RL-71 which had shown a long period of effectiveness as an oral systemic in rabbits against both body lice and Aedes aegypti mosquitoes--and 24 other selected compounds were tested as oral systemics in guinea pigs against oriental rat fleas. Shell 52-RL-71 gave an extended residual effect similar to that found with body lice and mosquitoes. Of the 24 other materials, 11 compounds caused complete mortality of some lots of fleas within 5 hours after treatment without noticeably affecting the guinea pigs. Four compounds caused complete mortality of some lots of fleas within 5 hours after treatment but seriously affected or killed the guinea pigs.

Screening tests to find new repellents for application to clothing utilizing nymphal lone star ticks or chigger mites as the test species were continued. Of 109 new compounds tested against the nymphal lone star tick, 17 were rated as Class IV materials giving 80% repellency for periods ranging up to 50 days. Against chiggers none of 147 materials screened were effective enough to be rated Class IV materials.

With chigger mites the effect of aging on the effectiveness of deet impregnated in cloth was determined using 5 concentrations of deet. At 1.0 and 0.75 gms./sq. ft. deet remained effective for 6 days, at 0.5 and 0.25 grams for 5 days, and at 0.125 grams for 4 days.

PUBLICATIONS REPORTING RESULTS OF USDA AND COOPERATIVE RESEARCH

Basic Biology, Physiology, and Nutrition

Bigley, W. S. and F. W. Plapp. 1962. Metabolism of malathion and malaoxon by the mosquito Culex tarsalis Coq. Ins. Phys. Vol. 8, pp: 545-57.

Jay, Edward G., Jr. 1962. Species of Hippelates (Diptera: Chloropidae) gnats collected from mammals. Jour. Econ. Ent. 55: 1011-2.

Jay, Edward G., Jr. 1963. Organic matter requirements for the immature stages of Hippelates pusio Loew and related species. Jour. Econ. Ent. 56: 127-9.

Riley, Jack and Robert A. Hoffman. 1963. Observations on the meteorological-mosquito population relationship at Stoneville, Mississippi, 1959-60. Mosq. News 23: 36-40.

Woodard, D. B., D. E. Weidhaas, and H. R. Ford. 1962. A method to study mating activity of Anopheles quadrimaculatus Say under field conditions. Fla. Anti-Mosq. Assoc. 33: 24.

Insecticidal and Sanitation Control

Chapman, H. C. and L. L. Lewallen. 1963. Field tests with insecticides against mountain Aedes larvae in California. Mosq. News 23: 41-44.

Gahan, J. B., G. C. LaBrecque, and H. G. Wilson. 1962. Relative toxicity of selected compounds as residual sprays against adults of Anopheles quadrimaculatus Say. Mosq. News 22: 333-6.

Harris, Robert L. and Robert Henry Jones. 1962. Larvicide tests with colony-reared Culicoides variipennis. Jour. Econ. Ent. 55: 575-6.

Hoffman, R. A. 1962. Control of vectors--equipment and insecticides. Proc. 4th Natl. Anaplasmosis Conf., Reno, Nev., pp: 84-5.

McDuffie, W. C., J. B. Gahan, A. N. Davis, and G. C. LaBrecque. 1962. Highlights of recent research on mosquito control by the Entomology Research Division, USDA. Proc. 49th Annual N. J. Mosq. Exterm. Assoc. Meeting.

Reed, L. B., R. C. Bushland, and G. W. Eddy. 1962. Yearbook of Agriculture pp: 340-3.

Insect Sterility, Attractants, and Other New Approaches to Control

Dame, David A. and Claude H. Schmidt. 1962. The importance of competitiveness of radiosterilized males in mosquito control programs. Proc. 49th Annual Meeting N. J. Mosq. Exterm. Assoc., pp: 165-8.

Gertler, S. I., H. K. Gouck, and I. H. Gilbert. 1962. N-alkyl toluamides in cloth as repellents for mosquitoes, ticks, and chiggers. Jour. Econ. Ent. 55: 451-2.

Gilbert, I. H. and H. K. Gouck. 1962. Effect of insect repellents on plastics, paints, and fabrics. ARS 33-78.

Gouck, H. K. and Carroll N. Smith. 1962. The effect of age and time of day on the avidity of Aedes aegytpi. The Fla. Ent. 45: 93-4.

Gouck, H. K. and I. H. Gilbert. 1962. Responses of mosquitoes and stable flies to a man in a light-weight rubber diving suit. Jour.Econ. Ent. 55: 386-92.

LaBrecque, G. C., Carroll N. Smith, and D. W. Meifert. 1962. A field experiment in the control of house flies with chemosterilant baits. Jour. Econ. Ent. 55: 449-50.

LaBrecque, G. C., D. W. Meifert, and H. K. Gouck. 1963. Effectiveness of three propylenimine derivatives as house fly chemosterilants. Fla. Ent. 46(1).

LaBrecque, G. C., D. W. Meifert, and R. L. Fye. 1963. A field study on the control of house flies with chemosterilant techniques. Jour. Econ. Ent. 56: 150-2.

Plapp, F. W., Jr., W. S. Bigley, G. A. Chapman, and G. W. Eddy. 1962. Metabolism of methaphoxide in mosquitoes, house flies, and mice. Jour. Econ. Ent. 55: 607-13.

Weidhaas, Donald E. 1962. Chemical sterilization of mosquitoes. Nature 195(4843): 786-7.

Weidhaas, Donald E. and Claude H. Schmidt. 1963. Mating ability of male mosquitoes, Aedes aegypti (L.), sterilized chemically or by gamma radiation. Mosq. News 23: 32-4.

Weidhaas, Donald E., Claude H. Schmidt, and E. L. Seabrook. 1962. Field studies on the release of sterile males for the control of Anopheles quadrimaculatus. Mosq. News 22: 283-91.

AREA NO. 18. BEES AND OTHER POLLINATING INSECTS

Problem. The pollination of some 50 seed and fruit crops depends on an abundance of honey bees and other pollinating insects. Although certain wild bees help to pollinate cultivated crops, honey bees are estimated to account for three-fourths of the pollination by insects. Most growers are not fully aware of the importance of honey bees and the wild bees in the production of pollinated insect crops. Therefore, research that leads to more efficient and economical production of honey bees is imperative to insure effective pollination of many crops and the economical production of honey. A problem of major significance is the increasing use of pesticides, many of which are hazardous to bees or destroy important pollen and nectar sources. There is need for more knowledge of the management of bee colonies; breeding of improved hybrid bees; physiology and behavior of queens, drones, and workers; and the various diseases and pests of the honey bee and means for their control. There is also need to study the many facets of the complex pollination problem to integrate effectively populations of honey bees and other pollinating insects with crop needs and practices. More knowledge should be obtained about wild insect pollinators and their management. It is also essential to study the effects of farm practices, such as the use of different pesticides, changes in crops, soil management, and harvesting, on the economy of the beekeeping industry and the survival of pollinating insects, and to develop procedures to minimize losses from such practices. Information is needed on nectar and pollen plants for use in conservation program efforts to provide bee forage areas in wastelands, watersheds, and roadsides. The nutrition of bees and the nutritive value of different pollens to bees require intensive investigation together with basic nutrition studies for development of pollen substitutes.

USDA PROGRAM

The Department has a continuing program involving apiculturists, geneticists, microbiologists, physiologists, and entomologists, engaged in basic studies and in research concerned with the application of known principles to the solution of crop pollination problems for the farmer and problems that affect the beekeeper. Bee breeding investigations at Baton Rouge, La., are cooperative with the State Experiment Stations of Louisiana, California, and Wisconsin, the Honey Bee Improvement Cooperative Association, and the Ontario Agricultural College, Guelph, Ontario, Canada. Bee management investigations at Madison, Wis., are cooperative with the Wisconsin and Arizona Experiment Stations, the Honey Bee Improvement Cooperative Association, the Department of Apiculture at Ontario Agricultural College, Canada, the Abbott and Pfizer Laboratories, the Eastern Utilization Research and Development Division, and the Agricultural Engineering Research Division. Investigations on bee diseases are carried on at Beltsville, Md., and Laramie, Wyo., in cooperation with the Louisiana, Wisconsin, and Wyoming Experiment Stations. Honey bee pollination investigations at Tucson, Ariz.,

are carried on in cooperation with the Experiment Stations of Arizona, California, Louisiana, Utah and Wisconsin. Wild bee pollination investigations at Logan, Utah, are conducted in cooperation with the Experiment Stations of Arizona, Utah, Louisiana, Wyoming, Idaho, Oregon, Washington, the Crops Research and Agricultural Engineering Research Divisions, ARS, and private beekeepers and farmers.

The Federal scientific effort devoted to apiculture research totals 22.1 professional man-years. Of this number 6.7 is devoted to breeding and management to improve productivity in honey bees; 4.7 to biology and control of diseases and pests of honey bees; 5.5 to behavior and utilization of honey bees and other insects in crop pollination; 2.7 to effect of pesticides and other farm practices on honey bees and other pollinating insects; and 2.0 to program leadership.

P. L. 480 grants total 9.25 man-years. Bee breeding research is being conducted under P. L. 480 funds at the Central Apicultural College, Warsaw, Poland (2.5 man-years) and at the Faculdade de Filosofia, Ciencias e Letras de Rio Claro, Rio Claro, Sao Paulo, Brazil (3 man-years). Bee disease research under P. L. 480 funds is underway with the Government Agriculture College and Research Institute, Ludhiana, Punjab, India (3 man-years) and with the Instituto Nazionale di Apicoltura, Bologna, Italy (3/4 man-year).

REPORT OF PROGRESS FOR USDA AND COOPERATIVE PROGRAMS

A. Breeding and Management of Honey Bees

1. Genetic Studies. Crosses between gentle and vicious lines of bees were tested in colonies and nuclei. Studies at Baton Rouge, La., indicated that temper in bees is probably determined by several genes with additive effects.

At Baton Rouge, three chemosterilants, tepa, metepa and apholate were fed to fertilized laying queens in mailing cages with attendant bees in concentrations of 0.1, 0.5, and 1.0% in a sugar solution for 1 or 2 days. All queens soon died except three fed metepa at 0.1, 0.1, and 0.5%, respectively. Oviposition was delayed when these queens were first reintroduced to their hives and hatchability of eggs was greatly or completely reduced. In all cases oviposition and viability to emergence was back to normal by the 48th day. Three colonies were fed 400 cc of 0.5% solutions of the three chemicals. The queens in colonies fed tepa and apholate laid sterile eggs to the ninth and tenth days, respectively. Then hatchability of the eggs and viability of the brood gradually increased until recovery was complete after 48 days. Metepa reduced viability of the brood only to 60% and recovery was also complete in 48 days.

Various stocks of bees tested for resistance to DDT at Baton Rouge differed only slightly in LD 50. A resistant stock from the Citrus Experiment Station at Riverside, Calif., was less resistant than some of the others

tested and two generations of selection have not materially increased resistance in this stock. Methods for testing individual worker bees as well as queens and drones have been developed, which give more repeatable results than the former method of testing many bees in a cage.

At Baton Rouge, eight lines of honey bees were tested for variances in egg weight and numbers of eggs laid by queens. Highly significant differences between lines were found in both variables. The environmental factors -- colonies, groups and weeks -- contributed significantly to the number of eggs laid by individual queens. There was no significant correlation between the number of eggs laid by indiviaul queens. There was also no significant correlation between the number of eggs laid and the weight of these eggs.

Bee breeding research is being conducted under P. L. 480 funds at the Central Apicultural College, Warsaw, Poland (E21-ENT-7). This work has shown that in an inbred colony with brood having about 50% viability all the eggs hatched and then about 50% of the larvae disappeared from the cells within 24 hours. Histological investigations showed that the larvae which disappeared were diploid drones produced from fertilized eggs. Thus, it was shown for the first time that diploid drones can develop from fertilized eggs. It was further shown that diploid drone larvae were eaten by the bees while the larvae were still alive. Some of these larvae were reared in an incubator without the attendants and more than one half of them were still alive after 2, 3 or more days, and more than one half of them were alive after 5 days. Research on bee breeding is also being conducted at the Faculdade de Filosofia, Ciencias e Letras de Rio Claro, Rio Claro, Sao Paulo, Brazil (S3-ENT-1). Significant findings on this project have been: An average of 1.38 lethal equivalents per bee was found in Apis mellifera mellifera and total mutation rate for deleterious genes is 7.5%; the dying larvae of 50% viable brood A. mellifera are females; queens of stingless bees are regularly fed with (in order of importance) worker eggs, glandular food put in the alveolus, and food given by workers.

2. Hybrid Bees. At Madison, Wis., the (ZX)(M1) hybrid produced 40 pounds more honey than other hybrids in the same test. The respective average yields obtained from the six hybrids (ZX)(M1), (DyZ)(M1), (6Z)(M1), (6X)(M1), Starline, and Midnite, were 285, 201, 246, 212, 218, and 148 pounds of honey in excess of their 12-month consumption. The (ZX)(M1) and (DyZ)(M1) hybrids ranked superior to all others on the basis of temperament, behavior, swarming tendency, use of bur comb, fall weight of brood nests, and brood area measurements. The (ZX)(M1) hybrid is a superior stock and will be released to the industry as soon as the mechanics of release are worked out. The hybrid breeding research has been in cooperation with the Department of Apiculture, Ontario Agricultural College, and the Honey Bee Improvement Cooperative Association. A conference was held July 8-9 with Dadant & Sons, Hamilton, Ill., to discuss the possibility of a cooperative agreement to facilitate release of Government stock. A proposal was drawn up for consideration in the Department of Agriculture.

At Madison, Wis., ten inbred lines have been maintained, breeder queens produced, and many hybrid combinations of these inbreds made for prescreening tests to determine their combinability and colony characteristics. Outcrossed inbred queens are difficult for commercial queen breeders to use. Use of 3-way hybrid breed queens (6Z)(X) to establish vigor in the breeders (grafting mothers) appears to be a sound practice. Lines 6 to Z are of similar stock origin, and (6ZX)(M1) stock performed as well as (ZX)(M1) in prescreening tests. Backcrossing (ZX)(ZX) to obtain greater vigor in the breeding queens is also being studied.

3. Overwintering Queens. Substantial progress has been made at Madison, Wis., with the overwintering of caged queens in multiple queen storage colonies, but certain problems have not been completely resolved. Queens, once accepted by the bees, depend for their survival upon a large population of bees, protected from Nosema infection, with the caged queens and food supply organized so that the winter cluster cannot pull away from the queens under severe, low temperatures. The population must be composed of relatively young bees in late fall so that they can survive in suitable numbers until early spring.

4. Smoking Bees. At Baton Rouge, La., the effect of a social disturbance of the honey bee colony was measured during the honey flow period for the seasons 1961 and 1962. The weight gains of disturbed colonies were compared with those left undisturbed. Colonies disturbed gained weight on the day of treatment but less than colonies not disturbed. The differences in weight gains were highly significant.

At Madison, Wis., the use of smoke from ammonium nitrate fertilizer placed on the hot coals of a smoker is a practice of some beekeepers in management of colonies for honey removal, colony manipulations, and requeening operations. Adverse effects on colony populations often result but are overlooked. In both cage and field tests, longevity of the bees was reduced about 50 percent by this practice. Toxicity is apparently due to nitric acid and ammonia formed in the decomposition of ammonium nitrate.

5. Removing Bees from Supers. At Tucson, Ariz., propionic acid and anhydride mixtures were superior to water dilutions for removing bees from supers. Experiments at Madison, Wis., show that dilution of propionic anhydride with water to obtain dispersion is not necessary. Propionic anhydride was applied full strength to the fume chamber pad and the use of one fume chamber allowed the removal of 25 shallow supers in 30 minutes on a clear, sunny day at 80° F.

At Madison, Wis., a method of removing supers with dilute glacial acetic acid and smoke was more effective than propionic anhydride at temperatures below 60° F. A high-volume, low-pressure air blast was also effective in removal of bees from honey supers. Prof. Gordon Barrington, University of Wisconsin, Agricultural Engineering Department, proposed and provided equipment consisting of a portable motor generator and an industrial vacuum

blower unit. Over 500 supers were removed with this equipment. One-half to one minute was required for each super. The supers had consistently fewer bees left in them than by any other method, including those using repellents.

At Tucson, Ariz., methods of screening attractants and repellents have been improved. In tests with 84 compounds 10 showed some degree of repellency. Two materials were more repellent than propionic anhydride. Seven materials showed more attractancy, two of which were outstanding in this respect.

6. Watering Bees. At Madison, Wis., a sanitary water supply for bees was provided by 30 square feet of synthetic sheet sponge floated in a tank. No bees can gain access into the tank and sanitation can be maintained by sterilizing the sponge. A wheelbarrow equipped with a water vat and rack for supporting hive bodies proved very efficient for cleaning up bur comb without stimulating robbing. (Bees able to contact exposed honey in times of scarcity of nectar will ordinarily become very excited and stimulated to robbing with accompanying stinging).

7. Wax Melting. At Madison, portable infrared gas heaters were adapted for melting beeswax. With this method scrap wax can be rendered inside the building under all weather conditions while the solar extractor requires sunlight.

8. Honey Quality and Refrigeration. Small samples of each lot of honey extracted at Madison were graded, and duplicates placed at room temperature, under refrigeration, and in deep freeze storage for observation of change. After 6 months of storage, samples at room temperature showed noticeable darkening and flavor change, while those under refrigeration and in the freezer did not. Samples under refrigeration all showed granulation starting, while those at room temperature or in the deep freeze had no granulation.

B. Diseases and Pests of Honey Bees

1. Diagnostic Service. At Beltsville, Md., 1,358 samples were diagnosed for diseases during 1962, of which 944 were brood and 414 adult bee diseases.

2. Tropilaelaps clareae. This mite is not known to occur in the United States. At Beltsville, brood comb samples received from the Philippine Islands and Hong Kong contained the mite, later identified as T. clareae. The mites were found in sealed brood cells containing honey bee pupae that were dead on arrival. The pupae had defective or deformed wings and under the pupae on the base of the cell were a number of adult mites, sometimes as many as 6 or 8. The mites were also dead on arrival. No mites have been located on or in the dead pupae. Whether the honey bee pupa is directly attacked by the mites is unknown. This mite is reported to be an increasingly serious problem to the beekeepers in the Philippines and Hong Kong.

Following suggestions of the Apiculture Research Branch, Ismael P. Sevilla of Malolos, Bulacan, Philippine Islands, has used Folbex as a possible control for this mite. His fumigation tries with methyl bromide, Tedion V-18, tobacco smoke and methyl salicilate were unsuccessful, and caging the queen to provide a broodless period did not control the mites. On the other hand, according to C. Y. Yip of Hong Kong, caging the queen for more than ten days during which period no brood is reared has successfully cleaned the combs of mites.

3. External Acarine Mites. At Beltsville, Md., the external mite, Acarapis dorsalis, was found on bee samples from Saskatchewan, Canada. Package bees shipped from Louisiana to Wyoming were infested with eggs and with immature and adult forms of A. dorsalis and A. externus mites.

4. Acarine Disease. No Acarapis woodi mites that cause the Acarine disease of honey bees have been found in the United States.

A survey made under P. L. 480 project A7-ENT-10 in India showed no A. woodi mites on about 100 colonies of Apis dorsata (giant honey bees) or A. florea (dwarf honey bees). A. woodi mites were found in 4 provinces on Apis indica (Indian honey bee). The occurrence of this mite in India is more widespread than was expected.

In the P. L. 480 project in Italy (E15-ENT-1) 3 of 15 materials tested proved to be nontoxic to bees and showed acaracidal effect of promise. They were: Chlorparacide, camphor + 5% safrol oil + 5% nitrobenzene, and champhor + 10% mustard oil. Examination of individual bees demonstrated that, when the infestation is very serious, the mites will spread extensively throughout the respiratory system of the thorax and the head (where they are more protected from the fumigant substances which constitute the traditional methods of control), instead of stopping in the prothoracic tracheae trunks, their habitual resting place. They may also transfer from the tracheae on one side of the thorax to those on the other side. The blood of healthy and infected bees revealed some differences both as to quantity and structure, which will be further investigated.

5. Gregarines. Gregarine parasites from the ventriculus of honey bees at Baton Rouge, La., appear very similar to specimens found several years ago at Laramie, Wyo., and St. Paul, Minn.

6. Virus Paralysis. At Laramie, Wyo., bee samples received from a colony showing paralysis in Washington State were crushed and filtered. A water suspension of the filtrate was either sprayed or fed in sugar syrup to healthy bees. Heavy mortality showed up on the 6th and 7th days among the sprayed bees and on the 9th to 12th days in fed bees. Uninoculated bees lived beyond the 23rd day. Symptoms of paralysis - crawling, wing flutter, paralyzed hind legs (but not loose hair) - were shown by dying bees. Tests of the sample for insecticides and protozoan diseases were negative.

A spore-forming bacillus and a small coccus were isolated from bees of a colony showing paralysis at the Madison, Wis., laboratory. When the coccus was injected into adult bees mortalities resulted in 24 hours without septicemia symptoms. The dead bees contained large numbers of the coccus. This coccus was highly sensitive to streptomycin.

7. Mycosis. In feeding tests at Laramie, Wyo., spores of the mold Aspergillus repens, in sugar syrup were not pathogenic to bees. The mold may occur as a saprophytic on dead bees.

8. American Foulbrood. At Laramie, 16 colonies with light AFB infections were fed at 4 weekly intervals with the following medicants: Sugar syrup with 1 gm./gal. sulfathiazol; sugar syrup with 1 gm./gal. terramycin; powdered sugar with 7 gm. of sulfathiazol; and powdered sugar with 7 gm. of terramycin. These treatments greatly reduced the amount of infection in the colonies. However, none of them completely eliminated the disease in all colonies. Four control colonies fed only unmedicated sugar syrup developed severe infections of the AFB disease. The disease also spread to 3 additional colonies not in the test.

At Beltsville, Md., in vitro tests with Tylosin lactate at the 0.1 p.p.m. level inhibited growth of Bacillus larvae. In vivo tests of Tylosin lactate at the 1,000 p.p.m. level was nontoxic to adult honey bees. Material toxic to brood and adult honey bees was found in a comb received at Beltsville from Virginia. The identity of the toxicant was not established. Bees and brood exposed to the pollen or to acetone extracts of it were dead in less than 18 hours.

Thiamine (vitamin B_1) is an indispensable growth factor for Bacillus larvae. Therefore a study was made at Beltsville to determine the part thiamine may play in the host-pathogen relationship of Bacillus larvae and the honey bee. Thiamine was tested by direct infection into the thorax and abdomen of adult honey bees to determine the degree of toxicity for the honey bee. No apparent toxicity developed when injections were made to the level of 30 micrograms of thiamine per bee. Therefore, additional tests were performed by injecting spores of Bacillus larvae plus thiamine at a level of 30 micrograms per bee at concentrations of B. larvae spores of 1,000,000, 100,000 and 10,000 spores per bee. The course of the infection resulting in mortalities did not appear to be affected by the presence of this additional amount of thiamine. The tests were repeated using an increased dosage of thiamine at the extremely high level of 10,000 micrograms per bee. No effect was apparent on either the course of the infection or the degree of mortality when the spore concentration was 1,000,000 per bee. However, at the level of 100,000 spores per bee, the infection did not develop and the percentage of mortality was reduced. This finding raises the question as to whether excessive amounts of vitamin B_1 would be helpful in protecting the honey bee from infection by B. larvae spores at dosages less than overwhelming.

Six package colonies developed American foulbrood during September at Madison, Wis. This disease was associated with equipment from diseased colonies of last year. One heavily infected colony was burned, and all colonies in the yard were treated with sodium sulfathiazole. Treatment will be continued in the spring. The equipment and five colonies concerned have been isolated, and progress of treatment will be closely observed to determine the effectiveness of preventative use of the antibiotic.

9. European Foulbrood. Two attempts to produce EFB at Laramie, Wyo., by spraying unsealed brood in 9 colonies with a water suspension of naturally infected larvae resulted in the removal of 25% of the larvae, but only 4 EFB diseased larvae were observed and the disease did not spread to the rest of the brood.

At Laramie repeated gorging treatments with sugar syrup containing 4 gms./ gallon of gallimycin cured 3 colonies with light EFB infections but not a colony that was heavily infected.

At Madison, Wis., outbreaks of EFB that commonly occur in May and June were prevented by April treatments, using 3 gorgings at 5 day intervals of syrup medicated with 100 mg. erythromycin activity or 150 mg. streptomycin per quart of syrup poured over the bees.

10. Spore-Forming Bacteria from Dead Brood. At Laramie, Wyo., larvae of three known age groups were inoculated individually, by contaminating the food in their brood cells with uniform sized droplets from a micrometer syringe with aqueous suspensions of the spores of one of three bacterial species isolated from dead honey bee larvae. Bacillus apiarius was recovered from only a single larva out of 600 inoculated with this species. B. pulvifaciens was not recovered from any of 600, or B. laterosporus from any of 300, inoculated larvae. The percentage of larvae removed before sealing, both inoculated and control larvae to whose food sterile water was added, varied tremendously in different tests. The percentage removal of 2-to 3-day old larvae usually was greater than that of either younger (1-to 2-day) or older (3-to 4-day) larvae in alternate rows of the same brood combs, inoculated on the preceding or succeeding days.

11. Sac Brood Disease. A sample brood comb (from Nebraska) containing an unusually severe case of sac brood, with at least 54% of the sealed brood diseased, was placed in the center of the broodnest of a colony at Laramie, Wyo., on May 1. The bees gradually cleaned up the disease, which remained negligible in the colony throughout the summer. By contrast, a moderate sac brood infection in a local colony moved to the same apiary at first nearly disappeared, then recurred to a serious extent. Three gorgings at 1-week intervals with 1 gram chloromycetin per gallon of 50% sugar syrup had no effect on the sac brood infection. The sac brood decreased naturally during the latter part of the brood-rearing season.

Approximately 3,800 brood cells were individually inoculated at Laramie with filtrates of naturally infected sac brood larvae passed through filter paper, Mandler porcelain filters, or Millipore filters. Honey bee larvae of three different age groups were inoculated with dilutions of the filtrates. Significant amounts of sac brood (12 - 75%) developed in only four out of 22 inoculated combs. Each of these was inoculated with a Millipore filtrate, showing that this disease is caused by a virus. Most of the filtrate-inoculated larvae were completely removed. Many of the water-inoculated controls were almost completely removed also.

12. Nosema Disease. The bee attendants in only 3 of 100 caged queens shipped from Georgia to Madison, Wis., showed no Nosema infection. Infections of 50% or more of the bees per cage were found in a third of the shipment. In commercial package bee shipments from the Southern States 40 of 75 packages showed Nosema infection. This is about the usual degree of infection occurring in such shipments.

Bee feeding tests at Laramie, Wyo., with aqueous suspension of Nosema spores resulted in 81% to 95% infection. The same suspension irradiated with ultraviolet light produced only a 1 - 6% Nosema infection. After one month of refrigeration the irradiated suspension was fed to bees and 11 days later the colonies were inoculated with Nosema spores. There resulted only 23% infection in these bees. In contrast sister bees fed with unirradiated suspension after 8 days developed 52 - 67% infection. Colonies fed only unirradiated syrup developed 85% Nosema infections.

At Laramie, queens held in mailing cages at room temperatures (70 - 80° F.) for two and a half weeks showed 83% Nosema infection when dissected, whereas queens from the same commercial shipment held at 92° F. were only 39% infected. Their worker attendants averaged 80% and 39% Nosema infections at the two temperature conditions, respectively.

At Laramie, queens confined over glass microscope slides during recovery from carbon dioxide anesthesia excreted a yellowish fluid on the slides which was readily examined for Nosema spores. None were found. Further study is required to evaluate the usefulness of this technique for demonstrating Nosema disease in living queens.

At Laramie, an aqueous suspension containing both Nosema spores and Amoeba cysts was poured in a thin layer on a filter paper between two glass plates. An electric current from a 6-volt battery was passed through the thin film of water by copper wires attached to each end of the filter paper. Material migrating to the anode or cathode after periods of 4 to 24 hours was pipetted from the respective poles, mixed with 60% sugar sirup, and fed to caged healthy bees. Under the conditions of the test, the two protozoan species were not separated by the electric current. Suspensions from either pole infected the bees to a similar extent with both parasites.

Caged bees fed a mixed inoculum of Nosema spores and Amoeba cysts at Laramie, developed 87% Nosema, 1% mixed infection, and no Amoeba infection alone. Bees began dying with Nosema infections 8 days after inoculation. The Nosema-diseased bees showed 50% mortality on the 20th day whereas uninoculated sister bees did not show 50% mortality until the 56th day. Thus, Nosema disease reduced the average longevity of caged bees by about 5 weeks.

Routine surveys at Laramie of 20-or 30-bee samples per colony for Nosema and Amoeba diseases were made at irregular intervals. Average Nosema infections ranged from 0 to 19% in the spring, and from 0 to 40% in the summer, in different apiaries. In late autumn, Nosema disease was found in only two colonies in only one apiary, and in about 1% of the bees of the infected colonies. It is suspected that this exceptionally low Nosema incidence for this locality may be correlated with unusually warm, dry, autumn weather. Low infections (5 - 10%) of Amoeba disease were found in two colonies in February, one colony in June (together with Nosema), and only one colony in December.

At Madison, Wis., the severe winter of 1961-62 prevented bee flight and caused higher than usual levels of Nosema infection. Monthly samplings were made from all overwintering colonies December through April. Seven percent of the colonies showed Nosema infection in December. By April, 28% of the colonies were infected. Foci of heavy infection in the yards were associated with queenless colonies that were unable to rear brood.

At a conference on Nosema disease the Canadians reported control of Nosema with fumagillin (Fumidil B) administered in dust form. Tests with package bees at Madison showed only a slight reduction in disease incidence from this treatment, but three repeated applications at 3-or 4-day intervals gave a measure of control. Fumagillin feeding was effective in controlling Nosema in queen storage and queen rearing colonies and nuclei. In November 1962, all colonies were fed 0.9 gallon of heavy sugar syrup (67% sugar) containing 100 mg. fumagillin per gallon. This treatment effectively reduced the initial Nosema in these colonies through the January samplings. Light Nosema infections were found in only two colonies through the January samplings.

13. Alkali Bee Brood Disease. Alkali bees in artificial nesting sites near Riverton, Wyo., were found to have a serious brood disease, even though mite infestations had been eliminated. No bacteria were evident in the diseased brood. At Laramie, it was suspected that the disease might be a virus infection similar to sac brood disease of the honey bee. Accordingly, a filter paper filtrate of diseased alkali bee brood macerated in water was used to inoculate individual brood cells containing 1 - 2-day old honey bee larvae. No disease appeared, and 97% of the inoculated brood developed into healthy bees, equivalent to that in water-inoculated controls. The virus may be specific to the alkali bee host Nomia melanderi.

14. Anthophora Bee Brood Disease. A single clay-like subterranean brood cell containing a diseased Anthophora bee pupa was sent to Laramie from Logan, Utah. Stained smears of the bee tissues failed to reveal any microorganisms.

C. Bee Behavior

At Madison, Wis., a reaction expressed by stopping a body activity or movement was identified in honey bees at a sound frequency range between 200 c.p.s. and 1,200 c.p.s. An optimum frequency of 960 c.p.s. was indicated. Amputation of the tarsi of pairs of legs and subsequent sound stimulation of the bee indicated that the forelegs are most important as a sound receptor or transmittor to the sensitive organ. Whether the sensitive organ is the scolopophorous organ or organs of the leg was not precisely determined. Leg contact with the substrate is an absolute necessity for sound reactions.

Research has been resumed at Baton Rouge, La., to find attractants in pollen. Most of the work to date has duplicated that which was originally done eight years ago. The following new information has been obtained: The attractive fraction in pollen is neutral, rather nonpolar, resistant to heat and ultraviolet light, acid and base stable for short periods, and deteriorates within 3 weeks in storage. Odor does not seem to be an attractant.

At Logan, Utah, in an attempt to determine the effect of stored-pollen reserves on pollen collection in confinement, only in a greenhouse cage did a pollen-poor colony collect pulverized pollen outside its hive. The pollen-rich colony showed no interest in collecting pollen under the same conditions. When the colonies were subsequently placed in a greenhouse cage, both eventually collected pollen; the pollen-poor one first and more avidly. Three colonies later brought into the bee room in February at Logan commenced pollen collection readily. It is postulated that in addition to pollen reserves, the duration of the dormant period before the bees were activated inside may have influenced the readiness of the bees to collect pollen.

At Beltsville, Md., for a period of approximately 6 weeks, an apparently normal queen laid a large percentage of multiple eggs in many brood cells. The multiple larvae were not removed after hatching but were tolerated in many instances through the mass feeding. When 3, 4, or 5 larvae were present per cell, these were generally reduced to 2 larvae which were tolerated until after the mass feeding period. Some of these larvae attained a weight of over 20 milligrams before removal from the cells. The surviving larvae in all instances went on to maturity and eventual emergence.

At Beltsville, an apparatus for extracting honey bee scent consisted of a closed chamber which would hold 4 pint mason jars and had a port through which a continuous stream of air could be introduced plus an exhaust port from which the air and the collected scent material could be exhausted. Approximately 500 honey bees were placed in this apparatus daily for a period of 5 weeks. The air recovered from this closed apparatus was passed

over a drying trap to remove moisture. The scent material obtained was then condensed on aluminum filings buried in dry ice. After each 5 days' extraction the condensed material was recovered from the aluminum filings by washing them with distilled acetone. Over 12,000 bees were exposed in this apparatus. The extracted material was turned over to the Pesticide Chemicals Research Branch for concentration and possible identification.

D. Utilization of Honey Bees and Other Insects in Crop Pollination

1. Sweetclover. At Madison, Wis., seed increase studies in cages with low coumarin sweetclover were carried out under 3 types of screen cover - 20-mesh saran cloth and 8-and 12-mesh fiberglass. The areas of the cages were 39,000 and 5,000 sq. ft. for saran cloth and 34,000 sq. ft. for fiberglass screen. Although bees worked well in all the cages, counts of bee visitors indicated the seed yields may range from high to low under 8-, 12-and 20-mesh in that order. Light reduction by the screen was apparently an important factor.

At Baton Rouge, La., a species of wild bee of the Halictine group has been identified as Lasioglossum versatum R. by Dr. Michener, University of Kansas. This bee is nesting in the ground on the campus of Louisiana State University and superficially appears to be the principal insect pollinator in the strawberry belt in Louisiana.

2. White Clover. At Baton Rouge, twenty colonies of honey bees were provided to pollinate from 20 to 25 acres of white clover (Trifolium repens) at the Idlewild Experiment Station. In earlier years there were no known white clover plants in pastures adjoining the station, but in 1963 some pastures within flight range of the colonies contained clover. Seed production continued to be unsatisfactory even though bee visits to the clover blossoms seemed normal. More than one half of the pollen collected in a pollen trap consisted of white clover pollen, showing that bees were visiting the clover blossoms. There were indications that trace elements (boron, copper, molybdenum, zinc) which had been applied to the soil by the experiment station caused an increase in the number of fertile florets and seeds per clover head. It is possible that pollination is adequate at Idlewild, but that soil conditions interfere with maximum seed production by the plant.

3. Cotton. Two Arizona Pima S-2 cottonfields were contracted for and supplied with bees for pollination in 1962. One field contained 100 acres and received 150 colonies of honey bees in 12 locations. The other contained 180 acres and was supplied with 180 colonies in 25 locations around the field.

At Tucson, data were taken from the 100-acre field. Over a 2-month period the average colony gain was only 34 pounds. The bee visitation to cotton was not satisfactory. Apparently the Pima S-2 cotton is less attractive to bees than the Pima S-1 variety previously worked with.

4. Safflower. At Tucson, an experiment was conducted in cooperation with Dr. Dave Rubis of the University of Arizona Agronomy Department to determine the effect of bees on the seed yields of two varieties of safflower. One was the normal commercial strain, Gila, while the other was a thin-hull selection developed by Dr. Rubis. The results showed that Gila safflower is capable of setting seed in the absence of bees. The thin-hull selection, because most of the anthers dehisce after stigma extrusion is complete, requires visits from bees to distribute the small amount of pollen that escapes the stigmal sheath. Safflower is highly attractive to bees; it is a good pollen source; and it produces a fair honey crop. The honey is thin, dark amber in color, and has a flavor that is not too desirable. The honey does not granulate even when left in the hive for a year. The nectar sugar concentration is about 20% as taken from nectar sacks of bees.

Bees visit safflower for nectar most of the day. Peak visitation is between 9 - 11 a.m. Pollen collection is mostly in the early morning. The Gila variety yields considerable quantities of pollen. Both Gila and thin-hull varieties are attractive to honey bees. Bees with pollen (presumably from the Gila variety) were observed visiting the thin-hull for nectar in Tucson.

At Tucson, the exclusion of bees in cages over the safflower plants reduced the seed yield by 50% in the thin-hull variety, but only 2.5% in the Gila, in comparison with the yield in cages containing bees. Caging (with bees) reduced the Gila variety yield by 15% over the yield of open-pollinated plots, but the reduction was slight with the thin-hull variety.

At Tucson, the oil content of seeds from the plots caged with bees, exceeded those of open plots by 2.1% with Gila, and 0.8% with the thin-hull variety. The increase in Gila would amount to 50 lbs. of oil/acre, which would be sufficient to make bees of value to the commercial producer for the benefit of oil content alone.

5. Red Clover. At Logan, Utah, observations were made on the pollinators visiting two experimental plantings of tetraploid and diploid red clover. Where the planting was isolated from colonies of honey bees and was surrounded by uncultivated land, bumble bees and species of Osmia were the most abundant pollinators. Many other pollinators were present but only in small numbers. Where the planting was not isolated from honey bees, these bees were the most abundant pollinators, but bumble bees, because of their greater efficiency, were probably nearly as important. Where diploids and tetraploids were side by side, yields were lower and differences in seeds per head between the varieties were not significant, perhaps because of complications caused by triploidy.

6. Alfalfa. At Logan, a total of 356 colonies from five apiaries in Box Elder and Cache Counties were checked for percentage of alfalfa pollen carried into the entrance. The sampling device developed for the purpose consisted of a motor and squirrel cage blower for suction connected with a

piece of vacuum tubing leading to a plastic centrifuge tube into which the bees arriving at the hive entrance were sucked. In the field a 12-volt automobile battery was used as a power source.

After checking all colonies on three dates, the five highest and five lowest colonies in terms of alfalfa pollen collection were selected and sent to the Baton Rouge, La., laboratory. Five or more queens bred from each stock (selected primarily on the basis of egg viability) at Baton Rouge will be sent back to Logan in the spring. From colonies containing these queens, the highest and lowest alfalfa-pollen-collecting colonies will again be selected and sent to Baton Rouge for further testing next year. It should be possible by this means to determine whether there is a genetic basis for alfalfa pollen collection.

7. Lipids in the Bee Diet. At Tucson, Ariz., the total and percent saponifiable and unsaponifiable lipids were determined in adult bees that had consumed diets of known fat content and compared with those of newly emerged bees not allowed to feed and older hive bees on their natural diets. The total lipid content did not differ significantly in bees fed fat-extracted and unextracted cottonwood pollen diets and was about equal to the total lipid content of bees fed sucrose candy. Bees fed unextracted dandelion pollen had a significantly higher total lipid content than those consuming other diets. Total lipids extracted from newly emerged bees had the highest percent of saponifiable material and the lowest amount of unsaponifiable substances. Lipids of bees fed sucrose candy had the highest percent of unsaponifiable material. The total lipid extracts of all bees analyzed without the digestive tract contained considerably more saponifiable than unsaponifiable material.

8. Wild Bee Pollinators. Taxanomic and biological studies of Anthidium (Hymenoptera, Megachilidae) were continued in 1962 and illustrations were completed for 27 species at Logan, Utah. Although in the same subgenus (Eutricharaea) and almost identical in size, shape, and color, M. rotundata and M. concinna are not difficult to distinguish in either sex. In the female the apical tergite of rotundata is more acurate in profile and has more erect pubescence. The male of rotundata has the teeth on the carina of the apical tergite mostly concentrated on an irregular, emarginate, median prominence, whereas in concinna the teeth are well separated and distributed around the carina, which is not particularly prominent medially. Also, the coxopodites of the male genitalia of rotundata have an apical process which is missing in concinna. The two species can also be distinguished by their leaf cells and cocoons. The leaf cells of concinna are more deeply telescoped into each other than are those of rotundata and the leaf pieces are more elongate and rectangular. The cocoons of rotundata are longer than those of concinna and the anterior end has the fecal pellets closer to the summit.

At Logan, observations were made on the nesting activity of M. rotundata including the effect of nesting materials, types of nests, straw sizes, color, movement of nests and temperature and humidity. Biology and ecology studies of a tomato pollinator, Anthophora occidentalis, demonstrated that the nesting season is primarily in July, that egg and larval development take about 15 days, that nesting occurs in vertical clay banks or walls, that thistle is the preferred host, and that tomato is quite attractive.

E. Effect of Pesticides on Honey Bees

Observations were made August 21-22 near Buckeye, Ariz., on the effectiveness of confining commercial apiaries under wet burlap tarpaulins to protect them, in this case, from methyl parathion and endrin sprayed by aircraft on 300 acres of nearby cottonfields. Colonies confined until 3 p.m., 4 hours after the applications were completed, suffered no mortality or apparent distress during the hours of confinement even though external air temperatures reached 104° F. Colonies left uncovered showed heavy mortality by 10 a.m. Within an hour after the bees were released to forage from the hives which had been covered they began to die in front of the hives and heavy mortality resulted. The bees were effectively protected during the period of confinement but not from the residual action of the insecticides. Length of time of confinement necessary for complete protection is unknown. It probably varies with different pesticides.

At Beltsville, Md., tests for toxicity to honey bees of solutions of various concentrations of new insecticides yielded the following results: ENT-25827, ENT-25828, ENT-25801, ENT-25831, ENT-25833, Bayer 47416, and Hooker HRS-1296, were less toxic than DDT to the honey bee; ENT-25819, ENT-25835, ENT-25788, Zectran, and ENT-28532 were more toxic than DDT but less toxic than malathion to the honey bee; ENT-25826, ENT-25923, ENT-25960, and ENT-25834 were more toxic than malathion to the honey bee.

At Beltsville, honey bees killed by various insecticides were extracted with organic solvents and the solutions examined under ultraviolet light after being spotted on filter papers. A definitely different pattern was revealed by the extracts from bees killed by chlorinated hydrocarbons, phosphates, or carbamates. However, this differentiation was true only when the bees were killed by an excessive amount of insecticide. When the amounts of the insecticides were reduced to an absolute minimum for mortality, differences were not obtained. This technique may prove of value for detecting honey bees killed by insecticides.

At Logan, Utah, bees from small observation colonies were trained to collect Sevin containing syrup from a feeder 25 to 30 yards from their hives. Individual marked bees made from 2 to 7 trips before becoming so affected that they no longer flew from the hive or were lost and presumed dead. They carried .08 to 1.6 µg. Sevin in syrup containing blue food color. Although the carrier bees usually recovered, hive bees receiving the syrup were often

killed. Bees trained to take untreated syrup while resting on a Sevin-treated surface made 12 to 59 trips before becoming seriously affected. Three bees exposed to residues of 1 pound per acre of Sevin recovered. One bee died and one recovered after exposure to 2-pounds-per-acre residues.

PUBLICATIONS REPORTING RESULTS OF USDA AND COOPERATIVE RESEARCH

Breeding and Management of Honey Bees

Nye, W. P. 1962. Extra supering and shading as factors in honey production in northern Utah. Gleanings in Bee Culture 90: 396-399, 436.
Farrar, C. L. 1963. Large-cage design for insect and plant research. ARS 33-77, 10 pp.

Diseases and Pests of Honey Bees

Moeller, F. E. 1962. Nosema disease control in package bees. Amer. Bee Jour. 102: 390-392.
Oertel, E. 1962. Behavior studies of the greater wax moth. Internationaler Kongress für Entomologie, Band 2, Wien.
Michael, A. S. 1962. Morphological characters of the honey bee mites. Bull. Apic. 5: 21-28, figs.
Michael, A. S. 1962. Tropilaelaps clareae, a mite infesting honey bee colonies. Bee World 43: 81-82.
Michael, A. S. 1963. Study of Acarine disease in Europe. Amer. Bee Jour., 103: 20-21.

Utilization of Honey Bees and Other Insects in Crop Pollination

McGregor, S. E., Alcorn, S. M. and Olin, George. 1962. Pollination and pollinating agents of the saguaro. Ecology 43: 259-267.
Alcorn, S. M., McGregor, S. E. and Olin, George. 1962. Pollination requirements of the organpipe cactus. Cactus and Succulent Jour. 34: 134-138.
Levin, M. D. 1961. Interactions among foraging honey bees from different apiaries in the same field. Insectes Sociaux 8: 195-201.
Nye, W. P. 1962. Management of honey bee colonies for pollination in cages. Bee World 43: 37-40.
Nye, W. P. and Bohart, G. E. 1962. Observations of the visits of honey bees and bumble bees to bladder senna (Colutea arborescens Lam.). Ann. Ent. Soc. Amer. 55: 259-260.
Pedersen, M. W. and Nye, W. P. 1962. Alfalfa seed production studies. II. Additional factors associated with seed yields. Utah Agric. Expt. Sta. Bull. 436. pp. 9-15.
Bohart, G. E. 1962. How to manage the leaf-cutting bee (Megachile rotundata Fabr.) for alfalfa pollination. Utah Agric. Expt. Sta. Circ. 144. 7 pp.
Bohart, G. E. 1962. Introduction of foreign pollinators, prospects and problems. Proc. Ist Internatl. Sym. Pollination, August 1960, Copenhagen. Published as Comm. No. 7, Swedish Seed Assoc. Pp. 181-188.
Bohart, R. M. and Bohart, G. E. 1962. A revision of the Larropsis subgenus Ancistroma Fox (Hymenoptera: Sphecidae). Proc. Ent. Soc. Wash. 64: 21-37.

AREA NO. 19. ANALYSIS, SYNTHESIS, FORMULATION AND EVALUATION OF INSECT CONTROL CHEMICALS

Problem. Modern insecticides are at present the most rapid and effective means of controlling injurious insects and their use has enabled the American farmer to produce an abundance of high quality crops and livestock. This extensive use, however, has been accompanied by increasing resistance of some insects to certain insecticides and by the possibility of leaving harmful residues on or in harvested crops, in meat, or in dairy and poultry products. There is therefore a need for the development of new types of chemicals, from natural sources and through synthesis, to which insects will not become resistant. These chemicals should be safe to handle and not leave harmful residues in the harvested products used for foods or feeds, or adversely affect wildlife, beneficial insects and other desirable organisms. More effective formulations of chemicals should be developed for the control of different insect species under various environmental conditions. Such chemicals and formulations require initial testing in the laboratory and evaluation under field conditions before they can be recommended for practical use. It is essential that accurate, sensitive analytical methods be developed for the determination of the amounts of chemicals deposited and the rate of disappearance of their residues and breakdown products in treated crops, animals, or soils. Better attractants are needed for use in traps and bait sprays for both insect detection and control. Research also is needed on repellents that would be useful in controlling insect attacks on crops, livestock, and man.

USDA PROGRAM

The Department has a long-term program supported by chemists, entomologists and scientists of other specialized disciplines to discover and develop new and improved insect control chemicals and methods of applying them. Chemical research to discover, isolate and identify products of natural origin which can be employed for insect control is carried on mainly at Beltsville, Md.; components of the cotton plant that serve as attractants or essential nutrients, or otherwise affect the boll weevil are being investigated at State College, Miss., in cooperation with the Mississippi Agricultural Experiment Station. Chemical research on synthetic organic materials and formulations for insect control is carried out at Beltsville, Md.; Gainesville, Fla.; and State College, Miss. Development of analytical methods for insecticide residues is carried on at Beltsville, Md. (headquarters); Tifton, Ga.; Vincennes, Ind.; Kerrville, Tex.; and Yakima, Wash. There is cooperation with the State Experiment Stations in the respective regions of all these laboratories. Cooperative work with the States on insecticide residues is carried on in connection with the following Regional Research Projects: NC-19, Fundamental Problems Associated with the Accumulation of Pesticidal Chemicals in Soils; NC-33, Pesticide Residues on or in Food, Feed, and Forage Crops; NE-36, Determination of Pesticide Residues on Raw Agricultural Commodities; S-22, Pesticide Residues on Plant and Animal Products and Soils; and W-45, Pesticide Residues, Their Nature, Distribution, and Persistence in Plants, Animals, and

Soils. Research on aerosols for insect control is conducted at Beltsville, Md. Biological evaluation of insecticides and other types of insect control chemicals is carried on at Beltsville, Md., and Brownsville, Tex. Research on methods for control of insects in aircraft is done at Beltsville, Md.

The Federal scientific effort devoted to research in this area totals 39.0 professional man years. Of this number 6.0 is devoted to products of natural origin as sources of insect control materials; 16.0 to development of synthetic organic materials and formulations for insect control; 5.0 to methods of analysis for insecticide residues; 2.0 to aerosols for insect control; 7.0 to biological evaluation of insect control chemicals; 1.0 to methods for control of insects in aircraft; and 2.0 to program leadership.

REPORT OF PROGRESS FOR USDA AND COOPERATIVE PROGRAMS

A. Products of Natural Origin as Sources of Insect Control Materials

1. Insect Sex Attractants. In the research carried on at Beltsville, Md., on natural insect sex attractants, the attractant produced by virgin female American cockroaches has been isolated and identified as 2,2-dimethyl-3-isopropylidenecyclopropyl propionate. This substance elicits a response from the males of the species in amounts less than 10^{-14} microgram. Attempts to synthesize the compound are in progress. Its structure is of a very unusual type which presents great difficulties in the synthesis.

In an investigation of the sex attractant of the pink bollworm, an extraction and chromatographic procedure has been developed at Beltsville which permits extraction of the whole bodies of the female bollworm moths, thus avoiding the time and labor required to clip off the abdomens of the moths.

In work with gyplure, the synthetic sex attractant for male gypsy moths that was discovered at the Beltsville laboratory, it has been found that some commercial batches may contain as many as 10 different constituents (byproducts and other impurities in addition to gyplure itself), whereas laboratory-prepared samples contain only 3 or 4. Methods have been developed for separating these different materials and determining the amount of pure gyplure in samples. The degree of attractancy depends on the purity, since the presence of more than small proportions of certain of the impurities results in inactivation of the attractant effect of the gyplure.

As an aid to the elucidation of chemical structures, a hydrogenolytic chromatographic procedure was devised for the interpretation of molecular configuration, using only a few micrograms of material. Called "carbon-skeleton chromatography," it has aroused great interest among the scientific fraternity. It proved very useful in establishing the structure of the American cockroach sex attractant.

2. Materials of Plant Origin for Insect Control. A material known as trans-Asarone that was isolated from Indian calamus oil at Beltsville has shown toxicity to adult body lice, Aedes mosquito larvae, and adult

mosquitoes in laboratory tests made at Orlando, Fla.

A sample of an extract of ground-up stems of Anona glabra (a wild plant in Florida) prepared at Beltsville for insect tests was sent to the National Institutes of Health, where it showed antitumor activity. Preliminary results of tests with different fractions of the extract indicated that the alkaloid fraction had the greatest antitumor effect.

At State College, Miss., a chemical investigation is in progress on the purification and identification of substances in the cotton plant that attract, arrest, or repel boll weevils.

B. Development of Synthetic Organic Materials and Formulations for Insect Control

1. Preparation of Synthetic Organic Compounds for Testing as Insect Control Agents. The research at Beltsville, Md., and Orlando, Fla., on insect chemosterilants, chemicals capable of suppressing the reproductive capacity of insects, is yielding promising results. The compounds that have been most consistently effective in inhibiting the reproduction of insects are the derivatives of aziridine (ethylenimine) and therefore they have received the greatest attention. A large number of new aziridines, both N-substituted and C-substituted, have been synthesized at Beltsville for evaluation at various field stations of the Division. In tests at Beltsville, apholate was an effective sterilant for male American cockroaches. A new compound of the dicarboxamide class that was synthesized was effective in sterilizing screw-worms in laboratory tests. A small quantity of radioactive tepa was synthesized for use in metabolism studies at Beltsville. At State College, Miss., the effect of some chemosterilants on the boll weevil is being studied.

Because of the importance of the aziridines, intensive studies have been made of their physical and chemical properties and new assay methods have been devised involving colorimetric tests, thiosulfate titration, paper chromatography, thin layer chromatography, and nuclear magnetic resonance. The last-named method is being used to investigate the stability of aziridines under various conditions of temperature and acidity. Since these compounds are unusually sensitive to acids, their sterilizing activity is dependent on the pH of the media, and careful attention must be given to their formulation. One method of formulation found useful for water-insoluble derivatives is emulsification with hydroxylecithin.

A special device has been constructed to separate male and female fruit flies (Drosophila) for chemosterilant studies. It uses a constant-velocity air stream to lift the lighter males into a separate chamber.

In cooperating with the Plant Pest Control Division operation to rid Florida of the Mediterranean fruit fly, a third isomer of the medfly attractant trimedlure was discovered. This isomer, which is completely unattractive to the medfly, comprised more than one-third of some lots of

the commercial lure purchased to detect the pest. A gas chromatographic procedure was developed for assaying the three isomers in trimedlure and a new purchase description was written to insure that the Plant Pest Control Division will obtain a superior lure in the future.

Considerable effort is being devoted to attempts to synthesize the American cockroach sex attractant referred to under heading A. The extremely unusual structure of this attractant molecule does not readily lend itself to synthesis. The dihydro analog of the attractant has been made, but it has no biological activity.

A number of new compounds were especially prepared as candidate attractants. Efforts were largely focused upon attractants for the European chafer, Mexican fruit fly, and medfly.

2. Formulations. Research on the development of improved formulations of insect control materials were conducted at Beltsville, Md., and Orlando, Fla. Two formulations were developed for the production of stable emulsions of the insect repellent deet, which is difficult to emulsify, especially in hard water. Simple procedures were devised for assessing emulsion stability.

At the request of the U. S. Army a proposed Federal Specification was drafted for an emulsifiable concentrate of deet suitable for field use to impregnate clothing and uniforms.

Gas chromatographic procedures have been devised for the analysis of two insect repellents, ethylhexanediol and deet.

Cooperation was extended to several national and international agencies interested in the improvement of pesticide dusts and powders. A nonwettability test was developed and proposed as an addition to the Federal Specification for 10% DDT powder. Three methods for evaluating the compactibility and flowability of dusting powders were reviewed and submitted to the Association of Official Agricultural Chemists. This study was also of interest to insecticide producers and to the World Health Organization. At the request of the Crops Research Division, the draft of a proposed specification for simazine herbicide dispersible powder and granular formulation was reviewed. Assistance was given to the U. S. Army Quartermaster in the development of a new type of oil-impregnated granules. At the request of General Services Administration the Interim Federal Specification for granulated aldrin was redrafted for conversion to regular Federal Status. The Interim Federal Specification for DDT water-dispersible powder underwent a similar revision. Physical tests were performed for the Plant Pest Control Division on the mirex bait used to control fire ants. Assistance was given to the U. S. Navy in connection with a DDT dusting powder having excessive wettability. In cooperation with the States of Oregon and Washington and the U. S. Forest Service a number of DDT-diesel oil samples were analyzed for conformance with specifications. Specimens of petroleum coke, wheat bran and corn cobs were evaluated as candidate

diluents. Polyethylene was found to improve the persistence of DDT and heptachlor sprays. It was shown that emulsified tung oil was suitable for the preparation of Sevin sprays to control gypsy moths.

3. Testing of Respiratory Protective Devices. Tests were conducted at Beltsville with respirators available commercially for protection against pesticides. A study was made of components used in filters and cartridges for respiratory protective devices. It was found that activated charcoal made from coconut shells is more efficient than charcoal made from petroleum residues. Filter masses made from a mixture of fibers are more efficient than those in which one type of fiber is used alone. An ARS publication and later a supplement to it were issued on protective respiratory devices. Copies have been in great demand from pesticide operators, growers, agricultural extension workers, the pesticide industry, public health workers, and others.

C. Methods of Analysis for Insecticide Residues

A method of analysis has been developed for the determination of residues of General Chemical 4072 in the fat and other body tissues of cattle. The method comprises extraction and clean-up of the sample and hydrolysis of the General Chemical 4072 with 12N sulfuric acid to produce 2,4-dichlorophenyl chloromethyl ketone, followed by gas chromatography of a hexane solution of the hydrolysis product.

An analytical method for dimethoate residues described in last year's report has been modified and adapted to the analysis of dimethoate in milk.

A colorimetric method that was developed in 1960 for the determination of coumaphos (Co-Ral) residues in animal tissues has been modified to make it applicable to the determination of coumaphos in dip solutions from animal-dipping vats. A fluorescence method has been devised for determining the presence of coumaphos on cattle hair; this provides a quick, easy means of determining whether cattle passing through screw-worm quarantine lines have been dipped in coumaphos.

A method was developed for determining ronnel in animal-dip solutions and on the hair of animals that have been dipped. The method involves hydrolysis of the ronnel to liberate 2,4,5-trichlorophenol and determination of the latter by ultraviolet spectroscopy.

The sulfide method of analysis for dioxathion (Delnav) has been modified to adapt it to the analysis of dips.

In cooperation with the Poultry Research Branch of the Animal Husbandry Research Division research has been continued to develop analytical methods for terephthalic acid (TPA) residues in chicken tissues. This acid is a synergist for certain antibiotics in treating chronic respiratory disease of poultry. A fluorometric method has been developed for this purpose and

and some data obtained on TPA residues in tissues of chickens fed a TPA-treated diet.

Another continuing emergency investigation being carried out at the request of the Pesticides Regulation Division concerns methods of analysis for chloroanilines produced by hydrolysis of detergents containing trichlorocarbanilide (TCC). Cases of cyanosis had been reported in premature babies wearing diapers that were laundered with TCC-containing products and autoclaved. Methods of analysis have been developed and it has been shown that chloroanilines are produced when solutions of certain detergents containing TCC are boiled. Diapers treated with a rinse additive containing TCC and then autoclaved contained about 35 parts per million of chloroanilines.

D. Aerosols for Insect Control

A U. S. public service patent (3,088,680) has been obtained for a dispenser for aerosols or similar pressurized products so designed that it will discharge the liquid contents when the container is held in any position. The usual aerosol dispensers will discharge the liquid only when the container is held upright. The new device is now being used on all pressurized ethylene oxide sterilizer units purchased by the Armed Forces.

A compact unit has been designed and constructed for testing the flammability of liquefied-gas-propelled aerosol formulations. Flammability tests have been made of a number of representative insecticide aerosol formulations of types that are frequently proposed to the Pesticides Regulation Division for label approval and registration and the results have been reported to that Division.

At the request of the World Health Organization tentative specifications have been drafted for household-type liquefied-gas-propelled insecticide aerosols and also for mist machines and thermal aerosol machines.

In cooperation with the aerosol industry a new lot of the Official Test Aerosol (OTA) formulation has been prepared and packaged for use during 1963-1965 as a standard for insecticide aerosol evaluation.

The stability of some organic phosphate insecticide aerosols has been investigated. An aerosol containing TEPP in methyl chloride, which was prepared in 1947, has recently been examined. Although the aerosol solution contained a white precipitate, it was still effective in killing aphids. DDVP in methyl chloride showed no loss in effectiveness over a 6-month period. DDVP-containing aerosol formulations will severely corrode aluminum containers but have little or no effect on tin-coated containers if the moisture content is low.

A small unit has been designed for sterilizing insect-rearing medium with a pressurized ethylene oxide sterilant formulation. The unit will hold six 4-inch petri dishes containing the medium.

Tests were made with various insecticide aerosol formulations dispersed by a thermal aerosol machine in greenhouses. The machine was a commercial Dynafog Model 70 that had been modified for use with small quantities of formulations. Applications of 1 gram of DDVP in 10 grams of methyl chloride per 1,000 cu. ft. by this means was very effective against mites and aphids.

A pressurized tree-injection apparatus has been developed in cooperation with the Crops Research Division. An isobutane-propane propellent mixture operates well. The back pressure in this type of treatment requires a special valve.

E. Biological Evaluation of Chemicals for Insect Control

1. Insecticides. A major activity in this area is the laboratory testing of synthetic organic compounds and natural products against representative species of insects to determine whether the materials have insecticidal, synergistic, attractant, repellent, insect chemosterilant, growth controlling, or other effects that would be useful for insect control. Preliminary evaluation tests on these materials are carried out at Beltsville, Md., and Brownsville, Tex., by the Pesticide Chemicals Research Branch and at 26 other locations by other Branches of the Division, throughout the United States and in Mexico, on 61 insect species and 9 mite species. Materials that show evidence of effectiveness are distributed to laboratories of the Division for more extensive secondary testing of their value. Some of the materials tested originate within the Pesticide Chemicals Research Branch and many others are supplied by other government or private research agencies and by industry.

Eight hundred eighty-five compounds and extracts or fractions of natural products were distributed for preliminary screening tests as insecticides during the year. The most effective of these materials were then distributed for secondary testing. Sixty-eight of the materials showed high toxicity to at least one species of insect or mite in laboratory tests.

A cooperative study is being made with the Insecticide Scientific Committee of the Chemical Specialties Manufacturers Association to develop a method for the evaluation of residual sprays against German cockroaches. Chlordane residues of 33 mg./sq. ft. deposited from a kerosene spray on glass and Masonite surfaces have given almost complete control of nonresistant roaches for 5 weeks.

2. Materials that Control the Activities of Insects Through Effects Other than Death. Increased emphasis has been given to the search for materials of synthetic or natural origin with properties that could be used to control insects by effects other than kill. One phase of this research is the investigation of insect sex attractants that are useful for detection of infestations and might be used for control by luring insect pests into traps or into contact with toxicants or sex sterilants.

Researchers at Beltsville developed a method for collecting and concentrating the sex attractant of the virgin female American cockroach. The crude attractant thus obtained was purified by the chemists and its chemical structure determined, as described under heading A. The course of the chemical work was guided by biological tests of the attractant activity of the various fractions obtained.

The female German cockroach also produces a sex attractant. The male German cockroach does not react as vigorously as does the American cockroach to its sex attractant and a different type of assay procedure therefore is being investigated.

Investigation of the sex attractant produced by the female southern armyworm moth has been continued. The insects are being reared at the Brownsville, Tex., laboratory and extracts prepared from the females for work on the isolation and identification of the attractant by the Beltsville chemists.

Large scale rearing of Cecropia moths is in progress at Beltsville to provide a source of insect juvenile hormone. This hormone prevents the development of insects to the mature stage and might have possibilities for control of insect pests. The material obtained from the moths is for use in a chemical investigation of the nature and composition of the hormone.

In connection with research on insect chemosterilants to prevent insect reproduction, a study has been made of the influence of pH on the effect of tepa (tris(1-aziridinyl)phosphine oxide) on fertility of house flies. The sterilizing effect on male flies was several times greater when the tepa was used in injection tests in alkaline solution than in acid. A laboratory study is in progress of the comparative effectiveness of tepa and apholate as chemosterilants for male house flies.

3. Aerosols and Space Sprays. Tests have been carried out with formulations of several of the newer insecticides in aerosol or space spray form to evaluate their effectiveness against resistant and nonresistant flies or mosquitoes. In connection with the preparation of the new lot of Official Test Aerosol for 1963-1965, referred to under heading D, biological tests were made to compare its effectiveness with the previous lot of this standard.

Several commercially available synergists for pyrethrins were compared for effectiveness in space sprays against resistant house flies. Sulfoxide was as effective as piperonyl butoxide, but propyl isome and MGK-264 were less effective. Sesame solids extracted from the seeds of the Northern Rhodesian black sesame plant, Sesamum angolense, synergized pyrethrins about two-thirds as well as did piperonyl butoxide against susceptible house flies.

Ten different carbamate insecticides were tested alone and in combination with piperonyl butoxide in space sprays against house flies in the laboratory. With two of these piperonyl butoxide showed a synergistic effect, with three an antagonistic effect, and with the remainder there was no marked degree of either synergism or antagonism.

F. Methods for Control of Insects in Aircraft

The method of aircraft treatment with DDT micronized dust to prevent the spread of Japanese beetles has been used by the Plant Pest Control Division for the past year in connection with Japanese beetle quarantine. Recent tests have indicated that a dosage of one gram of the 85% DDT dust per 1,000 cubic feet could be used satisfactorily instead of the two-gram dosage now in use.

Further cooperative tests have been carried out with the World Health Organization on the aerosol treatment of aircraft at "blocks away" (just after the door is closed for departure). Tests were conducted on airline flights in the Philippines and the Fiji Islands. The WHO standard reference aerosol containing pyrethrins and DDT with xylene and odorless petroleum distillate as solvents was compared with formulation G-1492, prepared by USDA and containing a higher concentration of pyrethrins with DDT and xylene. Both formulations were highly effective against nonresistant mosquitoes, but G-1492 was considerably more effective against resistant mosquitoes. G-1492 caused a little nasal irritation in passengers; it has been recommended, however, that this formulation be used where the presence of resistant insect strains has been proved.

Tests have been made with a pressurized formulation of ethylene oxide and propellents 11 and 12 against American cockroaches, confused flour beetles, resistant and nonresistant house flies, mosquitoes (Aedes aegypti), Japanese beetles, and grasshoppers. A dosage of $\frac{1}{4}$ lb. of ethylene oxide per 1,000 cu. ft. for 18 hours, 2 lb. for 3 hours, or 4 lb. for 2 hours killed 100% of adults and eggs of all the insects except the confused flour beetle, which required more than 48 hours. This is about 1/20 the dosage required to kill 100% of bacteria and other microorganisms.

In connection with some proposed experiments on the effects of radiation in space on insects, tests have been made on the irradiation of silkworm eggs with gamma rays. Eggs just out of diapause were much more susceptible to damage by the radiation than those in a later stage of development.

The effect from split radiation dosages was less than from the same total dosages given at one time. An insect-egg holder has been designed that utilizes photographic emulsion plates to permit the easy determination of heavy particle cosmic ray hits.

PUBLICATIONS REPORTING RESULTS OF USDA AND COOPERATIVE RESEARCH

Products of Natural Origin as Sources of Insect Control Materials

Jacobson, M. 1962. Sex attractants in survey and control of insects. Proc. North Central Branch Ent. Soc. of Amer. 17, pp. 8-9.

Jacobson, M., and Beroza, M. 1963. Chemical insect attractants. Science 140 (3574), pp. 1367-1373.

Jacobson, M., Beroza, M., and Yamamoto, R. T. 1963. Isolation and identification of the sex attractant of the American cockroach. Science 139 (3549), pp. 48-49.

Jones, W. A., Beroza, M., and Becker, E. D. 1962. Isolation and structure of sesangolin, a constituent of Sesamum angolense (Welw.). Jour. Org. Chem. 27, pp. 3232-3235.

Yamamoto, R. T., and Jacobson, M. 1962. Juvenile hormone activity of isomers of farnesol. Nature 196 (4857), pp. 908-909.

Development of Synthetic Organic Compounds for Testing as Insect Control Agents

Acree, F., Jr., and Beroza, M. 1962. Quantitative gas chromatography of insect repellent mixture M-1690. Jour. Econ. Ent. 55, pp. 469-471.

Acree, F., Jr., and Beroza, M. 1962. Quantitative gas chromatography of isomers of insect repellent N,N-diethyltoluamide. Jour. Econ. Ent. 55, pp. 619-622.

Alexander, B. H., and Beroza, M. 1963. Aliphatic amides of cyclic amines and tolyl maleimides as mosquito repellents. Jour. Econ. Ent. 56, pp. 58-60.

Beroza, M. 1962. Ultramicrodetermination of chemical structure of organic compounds by gas chromatography. Nature 196 (4856), pp. 768-769.

Beroza, M. 1962. Determination of the chemical structure of microgram amounts of organic compounds by gas chromatography. Analyt. Chem. 34, pp. 1801-1811.

Beroza, M. 1963. Trap for rotating evaporator. Chemist-Analyst 52, pp. 22-23.

Beroza, M. 1963. Identification of 3,4-methylenedioxyphenyl synergists by thin-layer chromatography. Jour. Agr. and Food Chem. 11, pp. 51-54.

Beroza, M., and Green, N. 1963. Materials tested as insect attractants. USDA, Agricultural Handbook No. 239, 148 pp.

Borkovec, A. B. 1962. Sexual sterilization of insects by chemicals. Science 137 (3535), pp. 1034-1037.

Feldmesser, J., Beroza, M., and Rebois, R. V. 1962. Effects of chemosterilants on saprophytic nematodes. Jour. Parasitol. 48, p. 44.

Fulton, R. A., Smith, F. F., and Busbey, R. L. 1962. Respiratory devices for protection against certain pesticides. U. S. Ent. Res. Div., ARS-33-76, 15 pp.

Fulton, R. A., Smith, F. F., and Busbey, R. L. 1963. Respiratory devices for protection against certain pesticides. U. S. Ent. Res. Div., ARS-33-76, Suppl. 1, 4 pp.

Gertler, S. I., Gouck, H. K., and Gilbert, I. H. 1962. N-Alkyl toluamides in cloth as repellents for mosquitoes, ticks, and chiggers. Jour. Econ. Ent. 55, pp. 451-452.

Gooden, E. L. 1962. An automatic sampling device for improved reproducibility in testing granular materials. Jour. Agr. and Food Chem. 10, pp. 397-399.

Hall, S. A. 1963. Pesticides seen as keynote to progress in U. S. agriculture. Croplife, pp. 50-52. Jan.

Hall, S. A. 1963. Insect attractants and chemosterilants. Proc. 16th Ann. Convention Assoc. Amer. Pesticide Control Off. Inc., Oct. 9-10, 1962, pp. 13-16.

Jacobson, M., and Beroza, M. 1963. Chemical insect attractants. Science 140 (3574), pp. 1367-1373.

Methods of Analysis for Insecticide Residues

Beck, E. W., Dawsey, L. H., Woodham, D. W., Leuck, D. B., and Morgan, L. W. 1962. Insecticide residues on peanuts grown in soil treated with granular aldrin and heptachlor. Jour. Econ. Ent. 55, pp. 953-956.

Brunson, M. H., Koblitsky, L., and Chisholm, R. D. 1962. Effectiveness and persistence of insecticides applied during the summer months to control oriental fruit moth on peach. Jour. Econ. Ent. 55, pp. 728-733.

Brunson, M. H., Dean, F. P., and Maitlen, J. C. 1962. Comparative effectiveness of different concentrations of spray in controlling codling moth and McDaniel mite on apple and pear psylla on pear. Wash. State Hort. Assoc. Proc. 58, pp. 61-64.

Brunson, M. H., Dean, F. P., Maitlen, J. C., and Butler, L. I. 1962. Control of codling moth and McDaniel mite on apple with a two-spray program. Wash. State Hort. Assoc. Proc. 58, pp. 67-69.

Butler, L. I., Maitlen, J. C., and Fahey, J. E. 1962. Microdetermination of Thiodan residues. Jour. Agr. and Food Chem. 10, pp. 479-481.

Claborn, H. V., Roberts, R. H., Mann, H. D., Bowman, M. C., Ivey, M. C., Weidenbach, C. P., and Radeleff, R. D. 1963. Residues in body tissues of livestock sprayed with Sevin or given Sevin in the diet. Jour. Agr. and Food Chem. 11, pp. 74-76.

Cleveland, M. L., Fahey, J. E., Hamilton, D. W., and Rusk, H. W. 1962. Evaluation of polybutenes as miticides. U. S. Ent. Res. Div., ARS-33-79 16 pp.

Cook, W. C., Butler, L. I., Walker, K. C., and Featherston, P. E. 1963. Granular in-furrow treatments with phorate and Di-syston against the pea aphid on peas. Jour. Econ. Ent. 56, pp. 95-98.

Derbyshire, J. C., and Murphy, R. T. 1962. Diazinon residues in treated silage and milk of cows fed powdered diazinon. Jour. Agr. and Food Chem. 10, pp. 384-386.

Fahey, J. E., and Hamilton, D. W. 1962. Thiodan residues on peaches. Jour. Econ. Ent. 55, pp. 563-564.

Giang, P. A., and Schechter, M. S. 1963. Colorimetric method for the estimation of dimethoate residues. Jour. Agr. and Food Chem. 11, pp. 63-66.

Jackson, R. D., and Fahey, J. E. 1962. Residues of EPN, endrin, parathion, and heptachlor on sweet corn treated for control of European corn borer. Proc. North Central Branch Ent. Soc. of Amer. 17, p. 151.

Still, G. W., and Fahey, J. E. 1963. Studies of insecticide residues on grapes and vines. U. S. Ent. Res. Div., ARS-33-81, 7 pp.

Walker, K. C., and Beroza, M. 1963. Thin-layer chromatography for insecticide analysis. Jour. Assoc. Off. Agr. Chem. 46, pp. 250-261.

Westlake, W. E. 1963. Pesticides. Analyt. Chem. 35, pp. 105R-110R.

Westlake, W. E., Corley, C., Murphy, R. T., Barthel, W. F., Bryant, H., and Schutzmann, R. L. 1963. Chemical residues in the milk of cows grazed on chlordan-treated pasture. Jour. Agr. and Food Chem. 11, pp. 244-246.

Aerosols for Insect Control

Fulton, R. A., and Yeomans, A. H. 1963. Aerosol flammability test method. Soap and Chem. Specialties 39(3), pp. 136-137, 142. (Also in Proc. 49th Ann. Meeting Chem. Specialties Mfrs. Assoc., Dec. 3-5, 1962, pp. 130-131)

Smith, F. F., Fulton, R. A., and Boswell, A. L. 1963. Some variations in response of two-spotted spider mite to acaricides. Jour. Econ. Ent. 56, pp. 224-227.

Biological Evaluation of Insecticides

Butt, B. A., and Cantu, E. 1962. Sex determination of lepidopterous pupae. U. S. Ent. Res. Div., ARS-33-75, 7 pp.

Fales, J. H., and Bodenstein, O. F. 1963. How to field test for cockroach susceptibility to chlordane. Pest Control 31(6), pp. 18, 20, 22, 62.

Jacobson, M., Beroza, M., and Yamamoto, R. T. 1963. Isolation and identification of the sex attractant of the American cockroach. Science 139(3549), pp. 48-49.

Keller, J. C., Johnson, V. A., Chisholm, R. D., Paszek, E. C., and Hill, S. O. 1962. Aerial spray tests with several insecticides against gypsy moth larvae. Jour. Econ. Ent. 55, pp. 708-710.

Yamamoto, R. T. 1963. Collection of the sex attractant from female American roaches. Jour. Econ. Ent. 56, pp. 119-120.

Methods for Control of Insects in Aircraft

Dutky, S. R., Schechter, M. S., and Sullivan, W. N. 1962. A lard-can device for experiments in photoperiodism. Jour. Econ. Ent. 55, P. 575.

Dutky, S. R., Schechter, M. S., and Sullivan, W. N. 1963. Monitoring electrophysiological and locomotive activity of insects to detect biological rhythms. Bio-Telemetry (Proceedings of Interdisciplinary Conference on the Use of Telemetry in Animal Behavior and Physiology in Relation to Ecological Problems), pp. 273-281.

Schechter, M. S., Dutky, S. R., and Sullivan, W. N. 1963. Recording circadian rhythms of the cockroach with a capacity-sensing device. Jour. Econ. Ent. 56, pp. 76-79.

Sullivan, W. N. 1962. Entomology in the upper atmosphere and outer space. Proc. North Central Branch Ent. Soc. of Amer. 17, pp. 6-8.

Sullivan, W. N., Azurin, J. C., Wright, J. W., and Gratz, N. G. 1963. Studies on aircraft disinsection at "blocks away" in tropical areas. WHO/Vector Control/19, 25 pp.

Sullivan, W. N., Schechter, M. S., Dutky, S. R., and Keller, J. C. 1962. Monitoring electrophysiological responses of cockroaches for space research. Jour. Econ. Ent. 55, pp. 985-989.

AREA NO. 20. IDENTIFICATION OF INSECTS AND RELATED ARTHROPODS

Problem. Only about a third of the estimated two million or more kinds of insects in the world have been described and named. Many of these are of no immediate concern to agriculture or mankind, but thousands of species are potentially destructive or useful. Minute morphological differences are very important in recognizing many species, and only highly trained specialists are able to positively identify known species and describe new ones. Precise information on the identity and distribution of insects is essential to the efficient conduct of programs concerned with research on harmful insects and the development of methods for their control, and in the management of regulatory activities intended to exclude, control, or eradicate insect pests.

Knowledge of the classification and identification of insects at present is far from adequate. Knowledge of the insect fauna of the world provides the best assurance that any potential pests will be immediately recognized, so that appropriate safeguards can be set up to exclude them or prompt action taken to control or eradicate them if accidentally introduced. Moreover, with increasing emphasis on the utilization of beneficial insect parasites and predators to help control destructive insects, it is necessary that we know which insects to search for, where they might be found, and how to recognize those that may be useful.

USDA PROGRAM

This program of the Department is a long-continuing one involving insect taxonomists, and includes basic research to make known to science previously unrecognized and undescribed species of insects, ticks and mites, and the application of results of this research to the problem of insect identification. The work is carried on to a limited extent at Beltsville, Md., but mostly in Washington, D. C., in close cooperation with the U. S. National Museum of the Smithsonian Institution. Somewhat less active cooperation is maintained with various centers of taxonomic research in the United States and foreign countries and with numerous individuals in many parts of the world.

The Federal scientific effort devoted to research in this area totals 30.0 professional man-years. Of this number, 6.8 is devoted to basic studies to name and describe beneficial and injurious insects, mites, and ticks; 15.4 to identification of insects, mites, and ticks; 6.8 to preparation of keys and monographs on the classification, distribution, morphology, and biology of insects and related arthropods; and 1.0 to program leadership.

Research in this area is also conducted under three P. L. 480 Projects. S9-ENT-6 (Uruguay) provides 1.5 professional man-years, F4-ENT-2 (Egypt) provides 4 professional man-years and A7-ENT-24 (India) provides 3 profes-

sional man-years. The Uruguay project is concerned with the classification of grasshoppers; the Egypt project with a survey of Egyptian insect fauna; and the India project (started in April 1963) with a systematic study of thrips. S5-ENT-2 (Colombia) has 2 man-years on Drosophila classification. A7-ENT-17 (Pakistan) provides 1.5 man-years on leafhopper taxonomy.

REPORT OF PROGRESS FOR USDA AND COOPERATIVE PROGRAMS

A. Basic Studies to Name and Describe Beneficial and Injurious Insects, Mites, and Ticks

1. Whiteflies. Whiteflies of the genus Trialeurodes are important economic pests and at least one species has been implicated as a vector of the yellow dwarf virus of sweetpotatoes. A list showing the numerous wild and cultivated host plants and the distribution of 5 of the most important species of the genus has been made available.

2. Biting Midges. For many years the most abundant and annoying biting midge in the mountains and woodlands of Northeastern United States and Canada has been known as Culicoides obsoletus. It has now been determined that there are 4 distinct species in eastern North America. These have been described and their known distribution and breeding habits reported.

3. Tetrastichus spp. Tetrastichus is one of the largest and most diverse genera in the Chalcidoidea. Many species are primary parasites, others are secondaries. Ten new species have been described adding considerably to the knowledge of this complex group.

4. Leafhoppers. Many species of leafhoppers are of great economic importance because they are vectors of plant diseases. Six new species and a new genus have been described from Central and South America.

5. Diptera Catalogue. A synoptic catalogue of the Diptera of America north of Mexico has been completed. It will be an indispensable aid to taxonomists working on Diptera just as the Hymenoptera Catalogue has been for specialists on that order. Preparation of the catalogue has taken a high proportion of the time of the dipterists working in this area.

B. Identification of Insects, Mites, and Ticks

Authoritative identifications and references to pertinent taxonomic and biological literature are supplied in support of Federal and State research, control, and regulatory activities pertaining to entomological problems. These services are also performed for industry, pest control operators, and private individuals in the United States and for foreign agencies and institutions concerned with entomology.

During the year, a total of 33,475 lots of insect material was received for identification. Well over 316,000 specimens were examined. A total of

84,377 identifications were made and reported. Material was not accepted for identification unless appropriate specialists were available to examine it or unless there was justification for the identifications. Nevertheless, the backlog of material awaiting study and report increased considerably.

The sources of material and the numbers of identifications made for each are shown in the following table:

Source	Number of Identifications	Percent of Total
Agricultural Research Service		
Plant Quarantine Division	22,095	26.19
Plant Pest Control Division	5,126	6.08
Entomology Research Division	4,107	4.87
Forest Service	1,544	1.83
Agricultural Marketing Service	870	1.03
Other Federal Agencies	1,953	2.31
States and Insular Possessions	21,532	25.52
U. S. individuals	21,520	25.50
Foreign agencies and individuals	5,630	6.67
Total	84,377	100.00

Many specimens received for identification represented species not previously in the National Collection, or they documented new distributional data. For these reasons 68,873 specimens were added to the Collection during the year.

The systematic review of technical literature essential to the programs in this area included the examination of 1,775 publications which contained 5,064 articles of scientific interest. A total of 2,031 articles was catalogued. From these 6,906 index cards to authors were prepared, and 15,498 cards were made up on which data of significance to taxonomists were recorded.

During the year 101 visitors obtained aid on taxonomic or nomenclatural problems. Some visitors remained only an hour or so, but many stayed one or two days and 8 remained for more than a week.

C. Preparation of Keys and Monographs on the Classification, Distribution, Morphology and Biology of Insects and Related Arthropods

1. Mites. The importance of phytophagous and predaceous mites as agricultural pests, carriers of diseases and as biological control agents can hardly be overemphasized. Several publications adding to available knowledge of their classification have been completed.

2. Moths. A revision of the family Cosmopterygidae and descriptions of new species of Perimede have added greatly to the knowledge of these tiny moths, many of which are leaf miners and of economic importance.

3. Two-winged Flies. An annotated list of Genus-Group names in the Family Simuliidae has been published. These black flies, or buffalo gnats, are vicious biters and serious pests. With present knowledge it is impossible to prepare a satisfactory world classification, but this publication shows the diagnostic characters of what appear to be nameable groups.

4. Beetles. Keys to the genera of the Tribes Tenebrionini and Ulomini, which include the mealworms and flour beetles, have been published. This information should aid considerably in the identification of these difficult groups.

5. Cockroaches. An illustrated report on the cockroaches collected on an expedition by Brazilian scientists into northeastern Brazil has been published in Portuguese (jointly with a Brazilian specialist). A number of little known and potentially important species was encountered.

6. Grasshoppers. Research under P. L. 480 Project S9-ENT-6 (Uruguay) on the collection, identification and classification of grasshoppers has progressed satisfactorily. An illustrated manuscript describing a male allotype has been prepared for publication, the male genitalia of about 25 species have been illustrated, and several collecting trips have been made.

PUBLICATIONS REPORTING RESULTS OF USDA AND COOPERATIVE RESEARCH

Basic Studies to Name and Describe Beneficial and Injurious Insects, Mites and Ticks

Baker, E. W. 1962. Tetranychus sinhai, a new species of mite from grasses (Acarina: Tetranychidae). Canadian Entomologist 94(9): 956-959, illus.

Baker, E. W. 1962. Description of the stages of chaetodactylus Krombeini, new species, a mite associated with the bee, Osmia lignaria Say. Proc. Biol. Soc. Wash. 75: 227-236.

Burks, B. D. 1962. A new Elachertus parasitic on a pest of bananas (Hymenoptera, Eulophidae). Ent. News 73: 233-235.

Burks, B. D. 1963. Ten new reared species of Tetrastichus (Hymenoptera, Eulophidae). Proc. Biol. Soc. Wash. 76: 47-58.

Gurney, Ashley B. and Maldonado C., J. 1962. On the cricket Hygronemobius alleni (Morse), with special reference to a first record of the genus in Puerto Rico (Orthoptera: Gryllidae). Caribbean Journal of Science 2: 156-158.

Jamnback, H. and Wirth, W. W. 1963. The species of Culicoides related to obsoletus in Eastern North America (Diptera: Ceratopogonidae). Ann. Ent. Soc. America 56: 185-198, illus.

Johnson, W. T. and Russell, Louise M. 1962. The black citrus aphid, Toxoptera aurantii (Fonscolombe) in Maryland (Homoptera: Aphidae). Proc. Ent. Soc. Wash. 64(2): 90.

Kramer, James P. 1963. New and little known Mexican and Neotropical Deltocephalinae (Homoptera: Cicadellidae). Proc. Biol. Soc. Wash. 76: 37-46, illus.

Krombein, Karl V. 1962. Natural history of Plummers Island, Maryland. XVI. Biological notes on Chaetodactylus krombeini Baker, a parasitic mite of the megachilid bee, Osmia (Osmia) lignaria Say. Proc. Biol. Soc. Wash. 75: 237-250, illus.

Krombein, Karl V., and Walkley, Luella M. 1962. Three hymenopterous parasites of an African mud-dauber wasp, Sceliphron spirifex (L.) Proc. Ent. Soc. Wash. 64: 78.

Krombein, Karl V. 1963. A new Nitelopterus from Wyoming and Washington. Ent. News 74: 61-63.

Krombein, Karl V. 1963. A new Chrysura from Plummers Island, Maryland. Ent. News 74: 149-152.

Millers, I., Benjamin, D. M., and Warner, R. E. 1963. A new Hylobius weevil associated with jack pine stand deterioration (Coleoptera: Curculionidae). The Canadian Entomologist 95(1): 18-22, illus.

O'Neill, Kellie. 1963. An oriental Taeniothrips (Thysanoptera: Thripidae) infesting certain Amaryllidaceae. Ann. Ent. Soc. America 56(3): 399-401.

Russell, Louise M. 1962. The citrus blackfly. FAO Plant Protection Bulletin 10: 36-38.

Russell, Louise M. 1962. The spittlebug Lepyronia coleoptrata (Linnaeus) in North America (Homiptera: Cercopidae). Coop. Econ. Ins. Rpt. 12(n. 16): 395-396, illus.
Russell, Louise M. 1962. New name combinations and notes on some African and Asian species of Aleyrodidae (Homoptera). Bull. Brooklyn Ent. Soc. 57(3): 63-65.
Russell, Louise M. 1963. Hosts and distribution of five species of Trialeurodes (Homoptera: Aleyrodidae). Annals of the Ent. Soc. America 56: 149-153.
Sabrosky, Curtis W. 1963. A new acalypterate fly from the tertiary amber of Mexico (Diptera: Milichiidae). Jour. of Paleontology 37: 119-120, illus.
Sabrosky, Curtis W. and Arnaud, Paul H. Jr. 1963. A holotype problem and a new specific name in Pseudochaeta (Diptera: Tachinidae). Ent. News 74: 155-156.
Smith, Marion R. 1962. A remarkable new Stenamma from Costa Rica, with pertinent facts on other Mexican and Central American species (Hymenoptera: Formicidae). New York Ent. Soc. Jour. 70(1): 33-38.
Smith, Marion R. 1962. A new species of exotic Ponera from North Carolina (Hymenoptera, Formicidae). Acta Hymenopterologica 1(4): 377-382, illus.
Spilman, T. J. 1963. The American genus Mycotrogus: a synopsis, a new species from Cuba, and a note on a larva (Coleoptera: Tenebrionidae). Proc. Ent. Soc. Wash. 65(1): 21-30, illus.
Steyskal, George C. 1963. Dichaetophora gracilis (Loew) (Diptera: Sciomyzidae) in England. The Entomologist 96(1196): 10.
Steyskal, George C. 1963. African Platystomatidae and Prygotidae (Diptera) in the Carnegie Museum Collections. Annals Carnegie Museum 36: 133-138.
Steyskal, George C. 1963. A second North American species of Traginops Coquillett (Diptera, Odiniidae). Proc. Ent. Soc. Wash. 65: 51-54, illus.
Stone, Alan. 1962. A new record for Parasimulium furcatum Malloch (Diptera: Simuliidae). Proc. Ent. Soc. Wash. 64: 174.
Todd, E. L. 1962. A new species of Eulepidotis Hubner from South America (Lepidoptera: Noctuidae). Proc. Biol. Soc. Wash. 75: 269-272, illus.
Walkley, Luella M. 1962. A second species of Ichneumonidae belonging to Scolomus Townes (Hymenoptera). Proc. Ent. Soc. Wash. 64(4): 231-233, illus.
Wirth, W. W. 1963. New synonyms in American Culicoides (Diptera: Ceratopogonidae). Proc. Ent. Soc. Wash. 65: 68.

Identification of Insects, Mites, and Ticks

Kingsolver, J. M. 1962. A note on techniques. Ent. News 73: 251.

Mattingly, P. F., Stone, Alan, and Knight, K. L. 1962. Culex aegypti Linnaeus, 1762 (Insecta, Diptera); proposed validation and interpretation under the plenary powers of the species so named. Z. N. (S.) 1216. Bull. Zool. Nomencl. 19: 208-219, illus.
Russell, Louise M. 1962. Aphis Linnaeus, 1758, its type species and the family-group name derived from it (Insecta, Hemiptera) Z. N. (S.) 881. Bull. Zool. Nomen. 19(pt. 4): 195-196.
Warner, Rose Ella. 1962. The scientific name of the alfalfa weevil (Coleoptera: Curculionidae). Coleopterists' Bulletin 16(4): 107-108.

Preparation of Keys and Monographs on the Classification, Distribution, Morphology, and Biology of Insects and Related Arthropods

Baker, E. W. and Pritchard, A. E. 1962. Macfarlaniella, a new genus of false spider mites (Acarina: Tenuipalpidae). Fieldiana-Zoology 44(15): 123-125, illus.
Dumrow, Robert, and Baker, E. W. 1963. The genus Nihelia (Acarina: Cheyletidae) V(2): 225-229, illus.
Hodges, R. W. 1962. The genus Perimede Chambers in North America north of Mexico (Lepidoptera: Walshiidae). Proc. Ent. Soc. Wash. 64: 145-154, illus.
Hodges, R. W. 1962. A revision of the Cosmopterigidae of America north of Mexico, with a definition of the Momphidae and Walshiidae (Lepidoptera: Gelechioidea). Entomologica Americana 42(NS): 1-171, illus.
Kramer, James P. 1962. New Liberian leafhoppers of the genus Recilia (Homoptera: Cicadellidae: Deltocephalinae). Proc. Biol. Soc. Wash. 75: 259-268, illus.
McDaniel, B. Jr., and Baker, E. W. 1962. A new genus of Rosensteiniidae (Acarina) from Mexico. Fieldiana-Zoology 44(16): 127-131, illus.
Pritchard, A. E., and Baker, E. W. 1962. Mites of the family Phytoseiidae from Central Africa, with remarks on the genera of the world. Hilgardia 33(7): 205-309, illus.
Rocha e Silva Albuquerque, Isolda, and Gurney, Ashley B. 1962 (November). Insecta Amapaensia -- Orthoptera: Blattoidea. Studia Entomologica (Brazil) 5: 235-256, illus.
Sabrosky, Curtis W. 1962. Insects of Macquarie Island (Diptera: Chloropidae, Milichiidae). Pacific Insects 4: 973.
Spilman, T. J. 1962. A few rearrangements in the Tenebrionidae, with a key to the genera of the Ulomini and Tenebrionini of America, north of Mexico (Coleoptera). Coleopterists' Bull. 16(2): 57-63.
Steyskal, George C. 1962. The genus Curranops Harriot (Diptera: Otitidae). Proc. Ent. Soc. Wash. 64: 117-118, illus.
Steyskal, George C. 1962. Notes on palaearctic Dryomyzidae, Helcomyzidae and Sciomyzidae (Diptera). Notulae Entomologicae (Helsinki). XLII: 71-72, illus.

Steyskal, George C., and Foote, R. H. 1962. Notes on the genus Icterica Loew in North America (Diptera: Tephritidae). Proc. Ent. Soc. Wash. 64: 166, illus.
Stone, Alan. 1963. An annotated list of genus-group names in the family Simuliidae (Diptera). Agr. Res. Serv. U.S.D.A. Tech. Bull. 1284: 1-28.
Wirth, W. W. 1963. Insects of Macquarie Island (Diptera: Ephydridae). Pacific Insects 4(4): 971.

General

Burks, B. D. 1962. Tetrastichus Walker, 1842, proposed suppression under the Plenary Powers. Bull. Zool. Nomencl. London 19: 306-307.
Gurney, Ashley B. 1963. A brief look at the Dismal Swamp and its natural history, especially the insects. Jour. Washington Acad. Sci. 53: 57-63, illus.
Krombein, Karl V. 1963. Behavioral notes on a Floridian mantid, Gonatista grisea (F.). Ent. News 74: 1-2, illus.
Sabrosky, Curtis W. 1962. Mating in Xylocopa virginica (Hymenoptera: Apidae). Proc. Ent. Soc. Wash. 64: 184.
Todd, E. L. 1962. In Beardsley Platysenta illecta (Walker), n. comb., notes and exhibitions of 662nd meeting, Feb. 13, 1961. Proc. Hawaiian Ent. Soc. for 1961, 18(1): 5.
Warner, Rose Ella. 1963. A rarity, Sphenophorus schwarzii Chttn., rediscovered (Coleoptera: Curculionidae). Coleopterists' Bulletin 17(1): 30-31.

AREA NO. 21. FOREIGN EXPLORATION, INTRODUCTION AND EVALUATION OF BIOLOGICAL CONTROL AGENTS

Problem. Many of the most serious insect and weed pests in the United States have been accidentally introduced from foreign countries without the insect enemies that kept them under control in their native homes. Some of the harmful insects so introduced have been effectively controlled by later introduction of their parasites and predators. Foreign exploration for beneficial biological control agents of insects and their subsequent introduction, colonization, and evaluation in this country is now a well established practice in the control of introduced insect pests. The use of imported insects to control introduced noxious weeds, although a more recent practice, has shown much promise. The biological approach to the control of insect and weed pests has great potential. Therefore, further foreign exploration is needed and additional research is necessary on the biology, ecology, nutritional requirements and the most effective manner of utilizing natural control agents, if they are to be used to maximum advantage. There is growing concern by the public over the insecticide and other residue problems in foods and by conservationists over the potential hazards of insect control chemicals to fish and wildlife. More effective use of natural control agents in meeting destructive insect and noxious weed problems could materially contribute to the ultimate objective of overcoming the pesticide residue and other hazard problems associated with the use of chemicals for the control of insects and weeds.

USDA PROGRAM

The Department has a continuing program on the use of beneficial insects. Basic and applied research is conducted on insect parasites and predators of insect pests and on insects that attack weeds, including foreign explorations for beneficial species and their introduction, liberation and evaluation in this country. A laboratory is maintained at Nanterre (near Paris), France, for studies on the parasites and predators of agricultural pests that have accidentally been introduced from Europe into the United States. At a station in Rome, Italy, studies are in progress on insects attacking a number of weeds, including puncture vine, Scotch broom, Dalmatian toadflax, Mediterranean sage, Russian knapweed, and halogeton. The field station at Rabat, Morocco, where studies on insects affecting halogeton had been conducted, was closed in March 1963, and the entomologist in charge transferred to the laboratory at Nanterre, France. Research on insects affecting aquatic weeds, especially alligator weed and water hyacinth, is being conducted at the National Agricultural Research Center in Castelar (near Buenos Aires), Argentina. Early in 1963 an entomologist spent about 6 weeks in Barbados collecting a predaceous beetle for release in Hawaii against the sugarcane mealybug. In the United States a receiving station and laboratory is maintained at Moorestown, N. J., where major emphasis is given to receiving, propagating and transshipping insect

parasites to proper liberation points. A laboratory for receiving, studying, and liberating insects affecting range weeds is located at Albany, Calif. Studies regarding entomophagous insects are also conducted at Riverside, Calif., and Beltsville, Md. The work at Albany and Riverside is conducted in cooperation with the University of California and the California Experiment Stations.

The Federal scientific effort devoted to research in this area totals 15.0 man-years. Of this total, 2.8 is devoted to search for and importation of foreign parasites and predators of insect pests; 2.1 to search for and importation of foreign insect enemies of weeds; 5.2 to basic biology, physiology, nutrition and evaluation; 3.9 to receipt, liberation and establishment of foreign insect enemies of insect pests and weeds; and 1.0 to program leadership.

Twelve grants for P. L. 480 funds have been executed for projects directly concerned with the study of insect parasites and predators. Nine of these projects involve exploration for beneficial species that might be shipped to this country for trial and release against agricultural pests here. These projects are as follows: Two projects - one in India (A7-ENT-5) and one in Pakistan (A17-ENT-8) - are for a study of parasites and predators of rice insects; two in the same two countries (A7-ENT-9, India, and A17-ENT-7, Pakistan) are for studies on parasites and predators of corn borers; one in India (A7-ENT-1) is for similar studies on sugarcane borers. Two projects - one in Pakistan (A17-ENT-5) and one in Poland (E21-ENT-2) - are for studies of the biological control of scales and aphids on fruit. Two projects, A7-ENT-2 in India and S9-ENT-1 in Uruguay are for surveys of crop pests and their parasites. These 9 projects call for 40 professional man-years annually. The other three projects directly concerned with studies of insect parasites and predators are A7-ENT-8, India (mass propagation), A7-ENT-17, India (rhinoceros beetle), and S3-ENT-7, Brazil (catalogue). These three projects call for 8 professional man-years annually.

Grants for two P. L. 480 projects have been executed for studies on the biological control of weeds. Project A17-ENT-9, Pakistan, is for a general survey of insects affecting noxious weeds, and Project A7-ENT-7, India, is concerned with studies of insects affecting witch weed. These two projects require 5 professional man-years annually.

REPORT OF PROGRESS FOR USDA AND COOPERATIVE PROGRAMS

A. Search for and Importation of Foreign Parasites and Predators of Insect Pests.

1. Parasites and Predators. A survey for natural enemies of the cereal leaf beetle, Oulema melanopa, in Italy, France and Germany indicated that several egg predators, 2 larval parasites and a larval disease are important factors in regulating outbreaks of the pest in central Europe. The same parasites that attack O. melanopa also attack O. lichensis, a closely related species more commonly found on wheat than on oats.

Thirteen species of parasites or predators of four different insect pests were collected in Europe; a coccinellid beetle predaceous on the sugarcane mealybug was collected in Barbados, B.W.I.; 11 species of parasites or predators from 5 insect pests were obtained through P. L. 480 projects in India, Pakistan and Spain; and a parasite of the brown soft scale was obtained from entomologists in Israel. All of this material was sent to Moorestown, N. J., for screening, testing, and transshipment to liberation points throughout the United States. In addition an introduced pea aphid parasite was sent to Colorado and Utah, and several parasites and predators of the black scale were sent to Iran directly from California.

B. Search for and Importation of Foreign Insect Enemies of Weeds.

1. Enemies of Weeds. Two insect enemies of weeds discovered in Europe appear quite promising and intensive tests are being made at Rome, Italy, regarding their host relationships. They are Apion fuscirostre, a seed weevil which attacks Scotch broom, and Phrydiuchus topiarius, a weevil which feeds in the stems and crown of Mediterranean sage.

C. Basic Biology, Physiology, Nutrition and Evaluation.

1. Alfalfa Weevil. A high proportion of the larvae of Bathyplectes curculionis, an imported parasite of alfalfa weevil larvae, are destroyed by phagocytes. In large alfalfa weevil larvae parasitized by only one Bathyplectes, phagocytes killed 54, 62, and 65% of the parasites in collections from New Jersey, southern California and northern California. When host larvae were parasitized by more than one Bathyplectes, comparable figures were only 23, 27, and 32%. Reasons for the apparent partial immunity and similar reactions in closely allied hosts are being studied at Moorestown, N. J.

2. European Pine Shoot Moth. Collections of European pine shoot moth larvae have been made in northern Germany at Lingen and in the Rhine Valley near Hartheim for the last 2 years. A tachinid fly, Lypha dubia, has been the dominant parasite at Lingen. It is heavily attacked by secondaries at Hartheim, but not at Lingen. There may be a correlation between secondary parasitism and the intensity of the shoot moth infestation which is increasing at Hartheim, but subsiding at Lingen. Lypha overwinters as a puparium. Studies are being conducted regarding the best way to hibernate the species under laboratory conditions.

3. Face Fly. Although about 34,000 face fly larvae were collected in the 9 Departments of France, no parasites were recovered from them or their puparia. A parasitic staphylinid beetle, Aleochora tristis, was recovered in small numbers from puparia obtained from cow chips utilized in a faunal study. The beetle does not appear very promising as a biological control agent, but its biology and habits are being studied. It can survive under very adverse conditions.

4. Cereal Leaf Beetle. A solitary, internal ichneumonid parasite, probably Thersilocus moderator, was the most important parasite of the cereal leaf beetle, Oulema melanopa, encountered in survey work in central Europe. Parasitization ranged from 12 to 77% at 12 collection points. Tetrastichus sp., a gregarious chalcid, which parasitizes Oulema larvae, was also important, with 44% parasitization recorded at one locality in Italy. A larval disease was prevalent and, where practical, farmers irrigate infested fields in Italy to increase the humidity and the incidence of larval disease.

5. Coccinella septempunctata. Studies are being conducted regarding the biology and food habits of the lady beetle, C. septempunctata, which appears to be a major factor in the control of the pea aphid and potato aphid in France. Two generations of C. septempunctata were reared between December 15, 1962, and March 13, 1963, in spite of the fact that some authors consider the species univoltine. An average of 800 eggs was laid per female. Cannibalism is a limiting factor.

6. Predaceous Wasps. Studies in France regarding predaceous wasps were continued with special emphasis on species nesting in sandy soil. Nests were excavated and the prey, plus the associated wasp egg or larva, was removed and held in an artificial cell covered with a glass slide. Details regarding larval development, construction of cocoons, and food requirements were thus determined for 12 different species.

7. Lygus spp. Studies regarding the parasites affecting lygus bugs are being conducted at Riverside, Calif., and limited observations of a similar nature are being made at Moorestown, N. J., and Paris, France. In southern California an egg parasite, Anaphes (Anagrus) ovijentatus, commonly parasitizes 50% of the eggs. A tiny braconid, Euphoriana uniformis, was reared from Lygus elisus in late summer when this species predominated, and from Lygus hesperus in late summer when this species became more numerous. Euphorus pallipes was reared from Lygus lineolaris in Moorestown and an undetermined Euphorus from Lygus rugulipennis in France.

8. Agasicles n. sp. Observations in Argentina indicated clearly that this multivoltine flea beetle, which is a suppressant of alligator weed in that country, does not necessarily go into diapause during the winter months. Consequently there should be no difficulty in synchronizing Agasicles n. sp. with seasons in the northern hemisphere, if it is introduced into this country.

9. Puncture Vine Weevils. The seed and stem weevils, Microlarinus lareynii and M. lypriformis, appear to be more effective suppressants of puncture vine in Italy than in France. The reasons for this are not clear. The parasites attacking the two species apparently are entirely different in the two countries.

10. Halogeton. Life history studies and intensive screening tests on Heterographis fulvobasella, insect enemy of halogeton, were continued at

Rabat, Morocco. Moths were attracted to black light in good numbers through September 10 thus providing an ample supply of eggs and larvae for feeding tests. The young larvae failed to survive on any test plants except halogeton and Mexican fireweed, indicating very strong host specificity.

11. Purslane. This weed, Portulaca oleracea, originally came to the United States from Europe or Asia. It is now widely distributed in this country and Canada. It is a pest of many irrigated crop areas in California. Studies are being conducted to determine the effect of the leaf mining sawfly, Sofus pilicornis, on the pest. The insect appears to be more active in the warmer inland areas than in the cooler, coastal region where the weed is most troublesome.

12. Thistles. Native thistles are much more severely attacked by native thistle head insects than is the introduced bull thistle, Cirsium vulgare. Feeding habits and life histories of the more common native thistle head insects are being studied in California.

13. Miscellaneous Insects. A list of 16 range weeds against which biological control work might be recommended has been compiled after a review of literature and a study of weed lists from 12 western States.

D. Receipt, Liberation and Establishment of Foreign Insect Enemies of Insect Pests and Weeds.

1. Alfalfa Weevil. The alfalfa weevil has continued its rapid spread throughout the East. Additional colonies of Bathyplectes curculionis from California, totalling about 5,000 adults, were liberated to hasten dispersion. About 9,000 Tetrastichus incertus were also released in the East, and 3,000 in California. This species was propagated in the laboratory. It is already established in the East and should prove valuable in California also. Bathyplectes anura from France was obtained in sufficient numbers for liberation for the first time. Forty individuals were released in New Jersey and 112 in Pennsylvania. The adult weevil parasite, Microctonus aethiops, was recovered in very encouraging numbers in New Jersey. A colony of 102 adults was released in New Jersey and 175 in North Carolina. Two hundred adults of Dibrachoides druso, a parasite which has not yet been recovered in this country, were supplied to the State of New Jersey for laboratory propagation and experimentation.

2. European Pine Shoot Moth. Releases of European parasites were continued against this serious pest in pine plantations. The following were collected in Germany and transshipped to the Forest Service in Michigan: 415 Pristomerus vulnerator, 612 Temelucha interruptor, 361 Orgilus obscurator and 379 Lypha dubia. Orgilus is established in this country. It is not yet known whether the other species have taken hold in Ohio and Michigan.

3. Brown Soft Scale. This pest of citrus remained at rather low population levels in Texas during 1962 due to high mortality caused by severe frosts

the previous winter. In 1963 it was again plentiful. Three species of parasites were propagated at Moorestown, N. J., and 944 Encyrtus lecaniorum, 660 Coccophagus cowperi and 1625 Aphycus sp., probably flavus, were sent to Brownsville, Tex., for colonization and experimentation. The first two species originated in Israel, and the last in California. Arrangements were also made to send a culture of the parasite Metaphycus stanleyi to Brownsville from the Citrus Experiment Station in Riverside, Calif.

4. Pea Aphid. Two parasites of this pest were released. Aphidius smithi, an important parasite from India which has become well established in California, was collected in that State and approximately 10,000 were sent to Colorado and 8,000 to Utah. Aphidius ervi was reared at Moorestown, N. J., and 225 adults were sent to Idaho, 275 to Washington and 485 to Belleville, Ontario, Canada.

5. Sugarcane Mealybug. This insect is of considerable importance in Hawaii. A predaceous coccinellid, Hyperaspis trilineata, is an effective control factor in Barbados. During January and February successful collections were made in Barbados and about 4,000 adult beetles were sent to Hawaii. Laboratory tests indicated that H. trilineata will also complete its development on the gray sugarcane mealybug which is sometimes abundant in sugarcane fields in Florida.

6. Balsam Woolly Aphid. Several balsam woolly aphid predators were collected in Europe and transshipped to the Forest Service via Moorestown as follows: 1,835 Laricobius erichsonii to New Hampshire, 942 Pullus impexus to Oregon, 779 Aphidecta obliterata to Oregon, and 800 A. obliterata to North Carolina. Laricobius is already well established in the major areas of infestation; Pullus is established in Oregon. Aphidecta is not positively established, although it is known to have successfully completed one generation in North Carolina.

7. Miscellaneous Insects. A few small lots of parasites were shipped for testing and release. Fourteen Opius rhagoletiocolus and 6 Phygadeuon wiesmanni from Switzerland and 120 Opius ferrugineus from New Jersey were sent to California for use against the cherry fruit fly. Twenty-eight specimens of a species of Aphidius from Europe were sent to Maine for use against the potato aphid. About 1,000 Cryptolaemus montrouzieri were sent to Iran from California for release against the citrus mealybug, and a consignment of black scales, expected to yield good colonies of Metaphycus helvolus, M. lounsburyi, M. stanleyi and Scutellesta cyanea, was sent to the same country from California.

8. Puncture Vine. The seed weevil, Microlarinus lareynii, has been successfully introduced into 6 western States for the control of puncture vine, Tribulus terrestris. In 1963 it was also successfully colonized in New Mexico, Texas and the Hawaiian Islands. On the island of Kauai it attacks a native Tribulus, T. cistoides as well as T. terrestris. T. cistoides is a perennial, and on this host the weevil apparently requires no diapause, for it has a year around food source.

9. P. L. 480 Projects. Investigations of parasites, predators and pathogens of sugarcane borers and corn borers are being conducted in India under P. L. 480 Projects A7-ENT-1 and A7-ENT-9. Two parasites of sugarcane borers were sent from India to the United States. Cocoons of Apanteles flavipes yielded 1,743 adults and puparia of Sturmia inferens yielded 156 adults. Both species were transshipped to the Grain and Forage Insects Research Branch laboratory at Canal Point, Fla., for study, propagation and possible release. Two parasites of Heliothis sp. were also sent to this country, but only one, an unidentified tachinid, was recovered as an adult. Fourteen were sent to Tifton, Ga., for testing against Heliothis zea.

Shipments of balsam woolly aphid predators were received from two Forest Service P. L. 480 Projects, A7-FS-7, India, and A17-FS-5, Pakistan. The following material from India was transshipped to the Southeastern Forest Experiment Station in Asheville, N. C.: 859 Leucopis sp., 487 Tetraphleps sp., 130 Chrysopa sp. and 22 hemerobiids. Shipments from Pakistan were also sent to Asheville. They comprised 487 Tetraphleps nr. pilipes and 322 Tetraphleps sp. Gypsy moth parasites from Spain, P. L. 480 Project E25-FS-10, were also handled for the Forest Service. Large numbers of puparia of the tachinid fly Tricholyga segregata were received. After the adult flies emerged, they were held for mating and then placed in suitable shipping containers for release in Connecticut by the Plant Pest Control Division. Up to June 30, 886 flies had been released.

10. Fall Webworm. Several lots of fall webworm parasites collected in this country were shipped to Yugoslavia. The fall webworm is now a serious pest in central Europe and it is hoped that American parasites will help to control it there. Collections of the fall webworm were made at several points in the United States and parasites reared from them in Arkansas by Dr. Tadic of Yugoslavia. The parasites were then sent to Moorestown, N. J., for shipment to Yugoslavia.

PUBLICATIONS REPORTING RESULTS OF USDA AND COOPERATIVE RESEARCH

Search for and Importation of Foreign Parasites and Predators of Insect Pests

Adlung, K. G. 1962. Observations on the occurrence of Tetrastichus turionum Htg. (Hym.: Euloph.) a parasite of Rhyacionia buoliana (Schiff.) (Lep.: Tortric.) (In German). Zeitschrift fur angewandte Entomologie 50(4): 455-462.

Basic Biology, Physiology, Nutrition and Evaluation

McGuire, J. U. and Sailer, R. I. 1962. A method of estimating face fly populations on cattle. ARS 33-80, 7 pages, 2 tables.

Janvier, H. 1962. Studies on nest building hymenopterous parasites of aphids. IV. The genus Diodontus. (In French). Ann. Sci. Nat. Zoology 12 Serie, Tome IV, fasc. 3, 481-516.

Receipt, Liberation and Establishment of Foreign Insect Enemies of Insect Pests and Weeds

Puttler, Benjamin and Coles, L. W. 1962. Biology of Biolysia tristis (Hymenoptera, Ichneumonidae) and its role as a parasite of the clover leaf weevil (Hypera punctata). Jour. Economic Entomology 55(6): 831-833.

General

Adlung, K. G. 1962. The effect of a DDT spray against the white fir budworm on the predators of the balsam woolly aphids. (In German). Zeitschrift fur angewandte Entomologie 50(2): 239-241.

Adlung, K. G. 1962. Occurrence of Bruchophagus spp. as pests of alfalfa and red clover in south and southwest Germany. (In German). Gesunde Pflanzen 15(3): 46-49.

Adlung, K. G. 1963. Collection of bark living insects. (In German). Ges. Mainz 2(2): 73-75.

AREA NO. 22: INSECT PATHOLOGY

Problem. Basic investigations on viruses, fungi, bacteria, nematodes and protozoa are needed to fully exploit the use of such microorganisms as an approach to insect control. There is much interest in the use of these natural insect-control agents to overcome the growing concern over chemical residues following the application of insecticides to agricultural crops and livestock, and the increasing resistance of some insects to certain insecticides. The utilization of pathogens to produce diseases in insect populations, and so reduce them and the damage they cause, is an approach that has already shown great promise. Microorganisms that are pathogenic for insects are generally very efficient when used properly. They are specific for their insect hosts and harmless to men and other vertebrates. Basic research is needed for a thorough understanding of insect pathogens, including their growth and nutritional requirements, their resistance to environmental factors, and their mutability and mode of action, both in the laboratory and the field. Such knowledge must be obtained before these organisms can be used effectively in the control of insect pests.

USDA PROGRAM

The Department's Pioneering Research Laboratory on Insect Pathology at Beltsville, Md., has a continuing basic research program on the growth, nutritional requirements and mode of action of viruses, bacteria and nematodes affecting insects. Studies are in progress on mutability-induced changes in virulence of insect diseases, and resistance of insects to diseases, including studies of the effect of the environment on the pathogens. A comprehensive reprint library on insect pathology is being assembled. Collections of all sporeformers and viruses known to cause disease in insects are being obtained from world-wide contributors. A service involving the diagnosis of unhealthy insects is now available to all Division, State, and University laboratories.

The program includes collaborative studies with the Pesticide Chemicals Research Branch on instrumentation for monitoring insect activity, internal temperatures of insects, and effect of gaseous atmosphere on metabolism and development of insects. Collaborative studies are also under way with the Pioneering Research Laboratory on Insect Physiology on the effect of microorganisms on insect sterol requirements and on the structural differences of the eye in Vitamin A deficient house fly adults as determined by the electron microscope. Cooperative studies with the U. S. Naval Missile Center on effects of cosmic radiation on pathogenicity and enzymology of insect pathogens and changes induced in their nucleoprotein make-up are under way.

A cooperative project has been conducted with the Fruit and Vegetable Insects Research Branch, involving the electron microscopy of the two-spotted mite. A second study with this branch has been initiated to study the

serology of the non-inclusion virus of citrus red mite, in order to develop a tool for diagnosis of the disease.

The Federal scientific effort devoted to research in this area totals 9.0 professional man-years. Of this number 2.6 is devoted to virus diseases of insects; 4.5 to bacterial, protozoan, and fungus diseases of insects; 1.0 to nematodes and their associated bacteria pathogenic to insects and 0.9 to discovery and study of new pathogens.

At Poznan, Poland (E21-ENT-6) research under P.L. 480 funds amounts to 1.5 man years annually and at Madras, India (A7-ENT-20), to 3 man years annually.

REPORT OF PROGRESS FOR USDA AND COOPERATIVE PROGRAMS

A. Virus Diseases of Insects

1. Polyhedrosis of Pine Sawfly, Neodiprion pratti-pratti. Tests of purified polyhedra freeze-dried on lactose showed that infectivity of these polyhedra was much lower (about 1/100th) than that of unpurified polyhedra from the tissues of freeze-dried whole insects stored for a like period of time. The difference in infectivity does not seem to be due to attenuation of the virus since fresh polyhedral suspensions prepared from larvae killed by both groups of virus were equally infective. As earlier studies showed that a pathogenic bacterium is also associated with the virus infection in this insect, it is possible that the reduced infectivity might be due to the elimination of the bacterium by the methods of purification employed. Pure cultures of the associated bacterium isolated in 1955 during the original studies of the disease and held on oil-flooded slants were viable and satisfactory for further study of the phenomenon in 1962. The strong fluorescense under ultra-violet light shown by this organism furnishes a simple means of recognition. Material from this purified polyhedral preparation was furnished to the late Mr. E. E. McCoy, New Jersey State Department of Agriculture, for tests of the virus against Neodiprion pratti-paradoxicus. The negative results obtained might have been due to the low titre of the virus material rather than the lack of susceptibility of the species. A retest of this species should therefore be made.

2. Granulosis Virus Disease of Red-banded Leafroller. The red-banded leafroller (Argyrotaenia velutinana) is now under culture in the laboratory to permit assay of frozen stocks of the virus now on hand and for propagation of virus in amounts sufficient for resumption of laboratory and field studies of the disease. Tests in 1956 and 1957 showed that the disease could be successfully established in apple orchards under field conditions. The studies now in progress will explore dosage-infection relationships, temperature limits, host range, and changes in blood proteins of the host caused by the disease.

3. Bacteriophage of Pathogen of American Cockroach. In the examination

of diseased cockroaches (Periplaneta americana) submitted by the Insect Physiology Laboratory, several pathogenic bacteria were isolated. Cultures of one of the isolates showed plaque formation and from the plaques a highly potent bacteriophage was recovered. Various filters were tested for their ability to pass the bacterial virus and to retain the host organism, and by means of a Morton filter bacteria-free active filtrates were obtained. Cultures of both susceptible and non-susceptible strains of the bacterium were also isolated. Injection tests demonstrated that both were pathogenic for wax moth larvae and that injection of the bacteriophage significantly reduced the effect of a challenging dose of the susceptible strain. After these preliminary studies were completed, cultures of the bacteria were made on oil-flooded slants and tubes of the bacteriophage were sealed and frozen to preserve the material for future study.

4. Abnormal Growth in Virus-infected Tissue. The previous annual report suggested the possibility of abnormal mitoses in fat cells of seventh instar larvae of the salt-marsh caterpillar infected with granulosis virus. It has now been established that no mitosis occurs in uninfected larvae in the last instar. However, excessive mitosis occurs in virus-diseased larvae, in fat cells adjacent to granulosis-infected cells.

It was postulated that a substance may be produced by infected cells that encourages mitosis in uninfected cells. Accordingly, water and chloroform ether (1:1) extracts were prepared from fat bodies of granulosis-infected, last instar larvae, and the water extracts were freeze-dried and concentrated in sterile water before injection into groups of uninfected last instar larvae. After two days, fat bodies of the injected insects were removed and prepared histologically for examination. There was no evidence of excessive mitosis. Consequently, no water-soluble material is produced by granulosis-infected fat cells that causes mitosis. The examination of the fatty materials is currently under way.

5. Virus Stock Culture Collection. Collection of insect viruses has continued. The laboratory now holds some 33 species of virus (several in substantial quantity, including the cabbage looper polyhedrosis, the red-banded leafroller granulosis, the salt-marsh caterpillar granulosis, and the Virginia pine sawfly polyhedrosis). Considerable effort is being made to obtain all known cytoplasmic polyhedroses since these viruses have a record of ability to cross-infect between related species of hosts. Attempts to infect the European corn borer with these microorganisms are under way.

B. Bacterial Pathogens of Insects.

1. Taxonomic Studies of Crystal-forming Bacteria. Twenty-eight separate isolates of crystal-forming bacteria including strains from Russia, Germany, Japan, France, Spain, Switzerland, Scotland, Kenya, Canada and the United States have been submitted to some thirty distinct biochemical, serological, and electrophoretic tests. Several discrepancies in the identification of

these bacteria have been detected and differences in toxin production are now known. A forty-eight-hour cell-esterase identification system has been established that should be of immense value as a diagnostic and epizoological tool. A paper of these findings is now in preparation.

2. Mode of Action of Crystal-forming and Non-crystal-forming Bacteria of Bacillus cereus and B. thuringiensis Group. (a) Bacillus thuringiensis var. thuringiensis and certain strains of this organism produce an exotoxin, the so-called fly factor, when grown on adequate media. This toxin can now be separated and purified by means developed at this laboratory in cooperation with the Insect Physiology Laboratory. The toxic component is strongly U.V. absorbent and exhibits a characteristic triple peak in the vicinity of 270 mu. These peaks are identical with Ca-dipicolinate suggesting that the non-toxic, pyridine salt is at least a marker for the toxin. Since the amount of 270 is proportionate to the biological activity in killing the house fly, an in vitro spectrophotometric test to determine the level of toxin in commercial preparations should be possible.

The fly factor interferes with pupation in the house fly Musca domestica, the face fly M. autumnalis, and the mosquito Aedes aegypti. The toxin is produced in cultures during sporulation or in the presence of dipicolinic acid, suggesting that a precursor of the toxin is formed before sporulation. This precursor may be harmful to young house fly larvae and the mode of action may be different from that which affects the prepupa of the house fly. Twenty-eight varieties of B. thuringiensis, B. entomocidus and B. finitimus were tested for exotoxin production. Only strains of B. thuringiensis (fourteen) produced the toxin. The remainder proved harmless to the house fly in tests involving some 12,000 larvae. According to other workers, this toxin can be given as a feed additive to cows and chickens and appear unchanged in the feces, preventing complete development of house flies in manure. This material, in this way, may be of considerable interest commercially. A paper on these findings is in the final stages of preparation.

In cooperation with the Apiculture Research Branch, preliminary tests were carried out with adult honey bees fed the fly factor toxin from Bacillus thuringiensis. At very high concentrations (200 mg. of crude exotoxin : 10 ml. of sucrose solution) the exotoxin proved fatal to the bees in 72 hours. This concentration is higher than could ever occur naturally in fields treated with B. thuringiensis.

(b) Cell-cementing substances in the midgut of insects susceptible to crystal-forming pathogens have been tentatively implicated, on histological grounds, as the pathogenesis of these disease agents. The mucoid cell-cementing substance of the midgut of Galleria mellonella has been isolated to further examine this hypothesis. The isolation procedure involved the removal of gut lipids in 4:1 ethanol chloroform followed by the solubilization of the remaining extraneous tissue by papain and a final trypsin, chymotrypsin proteolysis. A selective "salting out" procedure was used to separate the mucopolysaccharides that remained. This routine yielded a

fraction that corresponds to the polysaccharide, hyaluronic acid. Hyaluronic acid is, incidentally, one of the major cell-cementing substances obtained from vertebrates. Only traces of mucopolysaccharides were precipitated out of the insect material at salt concentrations used by other workers to obtain heparin and chondroitin sulfates. Preliminary analyses of the first fraction have established the presence of both uronic acids and amino sugars. The small amounts of the latter fraction have, to date, precluded their complete identification. Further extractions are in progress, and means to detect the break-down components of the hyaluronic-acid-like component in the infected insect gut are under investigation.

(c) Preparation of "clean" crystal preparations from crystalliferous bacteria are essential since investigators are now generally convinced that various strains of bacteria produce crystals of varying activity for different insect species. Several hundred milligrams of crystals have been produced during the year. Considerable amounts of these materials have been sent to laboratories in this and other countries to carry out cooperative studies.

Tests on the silkworm and the salt-marsh caterpillar with crystal preparations show that commercially produced materials are lower than expected in crystal toxicity. Crystal counts of these same preparations are approximately 1 crystal to two spores, which would not explain the lack of activity compared to laboratory produced material. This phenomenon is under investigation.

3. Clostridial Pathogens of Insects. (a) Extensive investigation of Clostridium malacosomae have been conducted, using most of the commercially available anaerobic media, in an attempt to propagate the bacterium. None of these experiments has led to a better medium than Bucher's. This completes the first phase of this investigation. An examination of the tent caterpillar gut contents is being conducted to detect and isolate the growth factor that permits growth of this fastidious organism.

(b) Since Bucher isolated three species of Clostridium from infected larvae of the tent caterpillar (Malacosoma pluviale), interest in the clostridia associated with insects has been stimulated. Special methods are required for isolating these fastidious pathogens from insects. Infected specimens are frequently grossly contaminated with aerobic and facultative bacilli. A device for the selective culture of clostridia has been developed. It is a simple compartmented culture tube consisting of an inner tube of borosilicate glass 7 mm. outside diameter and 115 mm. long. The inner tube is flared slightly at the top, flared and indented at the bottom, and perforated with four small holes about 25 mm. from the bottom. When the inner tube or separator is placed in a conventional culture tube containing liquid medium, two compartments are formed, one inside the separator and one between the separator and the culture tube. Infected insect material is delivered to the culture tube (outer compartment) with a long tipped Pasteur pipette. Isolations are made from the inner compartment to

fresh media. By this method, the isolation pipette does not contact the surface-growing aerobes in the outer compartment and permits enhanced and selective growth of clostridia from grossly contaminated material.

4. Bacterial Phagocytosis by Insect Blood Cells. The most prominent protective mechanism in insects is the phagocytosis of foreign bodies by certain types of blood cells. Some recent evidence indicates that the phagocytic activity of these cells may be increased following an intra-hemocoel injection of certain kinds of foreign materials. This suggests the formation of opsonin-like materials similar in function to those produced in mammals. Opsonins are antibodies which render microorganisms more liable to phagocytosis.

In vivo and in vitro tests are currently being conducted to compare the rate of phagocytosis of bacteria coated with immunized serum with bacteria coated with normal serum. Immune serum is prepared by injecting a group of insects with bacteria for a period deemed necessary for the production of the material in question. The blood is then extracted and centrifuged to remove the cells. If the presence of opsonins is established, electrophoretic separation and characterization may be attempted.

5. Effect of High Altitude Radiation on Spores of Insect Pathogens. The studies in cooperation with the Life Sciences Department, U. S. Naval Missile Center, were continued, and a broader protocol of study to include collaborative investigation of changes in the nucleoprotein make-up of exposed spores and their progeny was developed. A fourth payload (S/N A-6) of the BUWEPS HUGO III Program was successfully launched at Point Mugu, Pacific Missile Range on July 10, 1962, but again the payload was not recovered. No further launches were attempted under this program, and the program was terminated in September 1962. While no recovery was made under this program and the objective of the tests could therefore not be realized, much experience was gained that should be valuable in future studies.

A protocol based on the experience with the HUGO III Program was submitted in November 1962 as a biological experiment in space for the biological satellite program of the National Aeronautics and Space Administration. The experiment received favorable consideration but was deemed more suitable for high altitude balloon studies since these cheaper vehicles would provide adequate exposure and problems incident to recovery are much simpler. To obtain preliminary observations and to check on the methodology of the experiment, biopacks similar to those designed for the HUGO III Program were piggybacked in payloads of two high altitude balloon flights conducted by Carl E. Fichtel, Goddard Space Flight Center, NASA. The balloons, NASA Flight numbers 1040-N and 1042-N, were launched from Brownwood, Tex., on January 21 and 28, 1963. The first, aborted after 3 hours at altitude, and was recovered the following day at Roswell, N. Mex. Flight 1042-N completed the scheduled 6-hour flight at altitude and the payload was recovered minutes after touchdown at Odessa, Tex.

These tests, including laboratory and transportation ground controls, consisted of 24 biopacks. Of these, 6 biopacks were flown in each of the two flights, 3 containing spores of Bacillus popilliae and 3 containing spores of three strains of B. thuringiensis. All 24 biopacks contained eggs of Aedes aegypti in addition to spores of one of the bacterial pathogens. A few days after the return of the biopacks, unfortunate excessive temperature that occurred in the laboratory invalidated the test in respect to the mosquito eggs. Fortunately, the excess temperature did not affect seriously the nuclear emulsion monitoring plates and would have no effect on the spores. Hence valid results should be forthcoming in respect to the primary objective of these tests.

6. Rickettsiella popilliae. Studies with adult Japanese beetles show that the course of this disease in the adult is similar to that in larvae. The rickettsia attack only certain cells. These cells develop the typical blue coloration observed by dark field examination and contain numerous crystalline inclusions. Because of the opacity of the adult cuticle, the gross symptoms apparent in the larvae are not evident in the adult, but the microscopic picture is similar. At high dosages, examination of the blood removed by thoracic puncture shows large numbers of typically infected cells about 14 days after injection. The infected beetles are quite active at this time and are capable of normal flight. Diseased beetles are long lived and continue to feed until a few days before death. Females containing well developed eggs were dissected, and in spite of the enormous numbers of rickettsia present in the hemolymph, none were found inside the eggs. the beetles in these tests were not provided with soil, no eggs were deposited. An adequate number of tests were made with Japanese beetle adults to demonstrate that this stage could be used as an assay for viable infective rickettsia. The number of beetles available from field collection and their longevity decrease rapidly after peak emergence. Third-instar larvae reared from eggs in formaldehyde-treated soil were therefore used for this purpose.

Frozen tissue cultures, representing first and third passages of Rickettsia popilliae through human synovial cells, supplied by Earl Suitor, Virology Division, U.S. Naval Medical Research Institute, were thawed and broken up with and without sonication and injected at several dilutions into disease-free Popillia larvae. Portions of the frozen rickettsial suspensions used as the inocula of the tissue cultures were injected at several decimal dilutions so that dosages from 6 rickettsia to 6 million rickettsia per larva were obtained for comparison. A portion of this suspension was first sonicated to determine the effect of this treatment on the rickettsia. To conserve material, dosages employed with the sonicated material was from 6 to 600,000 rickettsia per larva. Sonication was performed at 1/10 dilution of the stock suspension, since 30 ml. of suspension was required. The results of these tests indicated but slight damage to the rickettsia due to sonication since 11 of 25 larvae injected with 6 sonicated rickettsia developed infection as compared to 25 of 25 injected with unsonicated material at the same level, whereas consistent infection was obtained with both suspensions at higher dosages. While positive infection was obtained with both tissue culture preparations at low dilutions (1/10 with culture No. 37-B, 1/100

with culture No. 43-1), the estimated numbers of viable rickettsia present in the cultures were very low and much less than those expected to remain by straight dilution of the large number of rickettsia (100,000,000) used as the original inoculum of the cultures. While it is possible that the rickettsia might have made some growth in the synovial cells, the amount of this growth would have been very slight, and would not constitute propagation of the agent. It is possible that larger numbers of rickettsia avirulent for the insect host might have been present in the cultures, but there is little microscopic evidence to support this. Typically infected larvae from all series have been frozen so that suspensions of rickettsia might be prepared and compared for infectivity by reinoculation at equivalent dosages.

C. Nematodes and Their Associated Bacteria Pathogenic to Insects.

1. DD-136 Nematode for Control of Codling Moth. The field test was continued in the Kearneysville, W. Va., orchard, in cooperation with entomologists of the Fruit and Vegetable Insects Research Branch, to determine whether applications of the DD-136 nematode would provide effective control of the codling moth. As in previous years, a very high degree of control was obtained in larvae under bands. Bark scraping of the trunks and scaffold limbs above the bands indicated that nearly equal control of larvae was obtained in these areas also. Nevertheless, a higher degree of fruit damage was experienced in young apples and at harvest than would be indicated from the degree of larval control attained. Fruit yields in the orchard in the fall of 1962 were high and in spite of the large number of apples on the trees at harvest, more than 40% of the picked fruit were wormy. The large population of larvae cocooning on the trunks showed over 75% nematode kill under bands by mid-October, and bark scrapings far up the scaffold limbs of sample trees showed a similar rate of kill under bark scales. The larval population was further reduced by early spring. A trunk survey in late April 1963 showed many old dead nematode-killed larvae, very few recently killed larvae, and a relatively small number of apparently healthy larvae. Two weeks following a nematode application in May 1963, nearly all overwintering survivors were found dead of nematode infection. Only a negligible number of the overwintering larvae in the orchard could have emerged as moths following this treatment. Nevertheless, an appreciable first brood infestation was present in the comparatively small number of fruits set. The large unsprayed orchard directly across the road from the test orchard and less than 250 yards removed from it had a fairly heavy load of apples last fall and undoubtedly contributed ovipositing moths to the test orchard this spring.

D. General.

1. Monitoring Electrophysiological Signals and Locomotor Activity of Insects. In cooperation with the Pesticide Chemicals Research Branch, the study of the electrophysiological and locomotor activity of the Madeira cockroach, Leucophaea maderae, to detect biological rhythms, has been con-

tinued and has now been submitted as a formal proposal for a biological experiment in space to the National Aeronautics and Space Administration for inclusion in its biological satellite program. The experiment is now under active consideration and counterpart experiments are being conducted at NASA's Ames Research Center at Moffitt Field, Calif.

A simple experimental infra-red detector system for recording and evaluating locomotor activity of house flies was constructed and demonstrated to personnel of the Orlando Laboratory, Insects Affecting Man and Animals Research Branch, for use in estimating the relative locomotor activity of chemically sterilized males versus normal males. The monitoring beam of infra-red was obtained by means of a Corning heat transmitting filter, and the test chamber is also provided with visible light that can be regulated to control the general level of activity.

2. Histopathology of House Flies fed Carcinogenic and Noncarcinogenic Analogues of 2-fluorenamine. In cooperation with the Insect Physiology Laboratory, histopathological examination of house fly larvae and pupae fed carcinogenic 2-fluorenamine derivatives revealed benign tumors similar to those hereditary tumors reported by other workers. Noncarcinogenic derivatives did not cause tumor formation. This suggests the possibility of using the house fly as a preliminary screening animal for carcinogenic materials. The large number of generations and sensitivity of these species would seem suitable for large scale testing operations. The tumors are usually found in the hemocoel and they consist of large rounded or polygonal cells scattered throughout a fibrous-like stroma. In older tumors the more centrally located cells become necrotic and are replaced by melanin deposits.

3. Histopathological Changes in Apholate-treated Drosophila. Histopathological examinations were made of gonadal tissues of Drosophila melanogaster adults previously treated with apholate and gamma radiation. In the treated testes there was a cessation of sperm production at the anterior end with a general necrosis of the germinal epithelium that progressed, in time, over the entire tubules. High level of either treatment halted production of viable eggs. Microscopic examination of Feuglen-treated ovarioles revealed very small Feuglen-positive clumps of chromatin, which indicated that complete breakdown of the nurse cells, oocytes, and follicle cells had occurred.

4. Electron Microscopic Investigation of the Structure of Rhabdoms of the House Fly Eye. Flies fed on a diet depleted of Vitamin A could perceive motion but not color. Biochemical and morphological studies by other investigators have indicated that Vitamin A is probably a component of the rhabdom wall structure. It was assumed that differences in this structure between control and Vitamin A free diet reared flies would be on the biochemical level or perhaps, if gross enough, might be detectable by electron microscopic examination.

Flies were reared aseptically on the following diets: (1) standard CSMA media, (2) CSMA media minus Vitamin A, and (3) CSMA media minus B-carotene.

Adult females were selected and amputations of eyes were performed in cold 2% osmium tetroxide fixative. Tissues were fixed 1-2 hours at 4°C., rinsed, dehydrated through an ethanol series and embedded in the following media: maraglas mixture C according to the method of Freeman and Spurdock; Epon according to Luft's method; and a methacrylate mixture (1 part methyl : 3 parts butyl). Difficulties were encountered in infiltration with each of the embedding materials used. Sections made from maraglas embedded tissues were stained with a saturated alcoholic solution of uranyl acetate to enhance the contrast. Methacrylate embedded tissues were stained with a 1% aqueous solution of uranyl acetate. Preliminary examinations have indicated that no gross morphological differences exist between rhabdoms from flies fed the control and Vitamin A free diet.

5. Investigation of Cuticle of Two-spotted Spider Mite. A cooperative project was initiated with the Fruit and Vegetable Insects Research Branch for the electron microscopic examination of cuticle from mites - nonresistant and resistant to organophosphates. Initially, whole female adult mites were fixed in osmium tetroxide, dehydrated and embedded according to standard techniques. The tissues observed were poorly fixed and inadequately infiltrated with the embedding medium. Next, mites were dissected on dry ice in the region of the cephalothorax, fixed in osmium tetroxide, dehydrated in an ethanol series, and embedded in a methacrylate mixture (90 parts butyl : 10 parts methyl).

The nonresistant mite integument is composed of an outer epicuticle, an exocuticle, a laminated endocuticle, a hypodermis, and a basement membrane and is morphologically similar to the insect integument. In many of the sections observed the cuticle was modified into cuticular extensions with lobed apices. These appear to be noninnervated and are undoubtedly similar to the immovable non-cellular processes already described on insects by other workers. Measurements of a few electron micrographs indicated the possibility of a difference in thickness in some of the cuticular components between resistant and nonresistant mites.

PUBLICATIONS REPORTING RESULTS OF USDA AND COOPERATIVE RESEARCH

Bacterial Diseases of Insects

Dutky, S.R. 1963. The milky diseases. Insect Pathology: An advanced treatise. Vol. 2, pp. 75-115, (Ed. E.A. Steinhaus) Academic Press, Inc., New York, N.Y.

Heimpel, A.M. 1962. Microbial insecticides. Proc. North Central Branch, Ent. Soc. Amer. 17, 80-84.

Heimpel, A.M. 1963. The status of Bacillus thuringiensis. Advances in Chemistry Series; New approaches to pest control and eradication. No. 41, 64-74.

Heimpel, A.M. and Angus, T.A. 1963. Diseases caused by certain spore-forming bacteria. Insect Pathology: An advanced treatise. Vol. 2, (Ed. E.A. Steinhaus) Academic Press, New York, N.Y.

Robertson, S.W., Jr. and Heimpel, A.M. Crystal preparations from commercial Bacillus thuringiensis var. sotto concentrates. J. Insect Pathol. 4, 274.

General

Adams, J.R., Holbert, P.E., and Forgash, A.J. 1962. Microscopy of contact chemoreceptors of the stable fly. N.Y. Light Microscopy Soc. Yearbook 8, 26-34.

Cantwell, G.E. and Henneberry, T.J. 1963. The effects of gamma radiation and apholate on the reproductive tissues of Drosophila melanogaster Meigen. J. Insect Pathol., 5, 251-264.

Dutky, S.R., Schechter, M.S., and Sullivan, W.N. 1962. A lard-can device for experiments in photoperiodism. J. Econ. Ent. 55, 575.

Dutky, S.R., Schechter, M.S., and Sullivan, W.N. 1963. Monitoring electrophysiological and locomotive activity of insects to detect biological rhythms. Bio-Telemetry. pp. 273-281, (Ed. Lloyd E. Slater) Pergamon Press, Oxford.

Schechter, M.S., Dutky, S.R., and Sullivan, W.N. 1963. Recording circadian rhythms of the cockroach with a capacity-sensing device. J. Econ. Ent. 56, 76-79.

Sullivan, W.N., Schechter, M.S., and Dutky, S.R. 1962. Monitoring electrophysiological responses of cockroaches for space research. J. Econ. Ent. 55, 985-989.

Wittig, G. 1963. Techniques in insect pathology. Insect Pathology: An advanced treatise. Vol. 1, pp. 591-636, (Ed. E.A. Steinhaus) Academic Press, Inc., New York, N.Y.

AREA NO. 23. INSECT PHYSIOLOGY AND MODE OF ACTION OF INSECTICIDES AND THEIR METABOLITES

Problem. Basic research in insect physiology is essential to the development of more efficient insecticides and new approaches to insect control. The increasing development of resistance to insecticides by insects has emphasized the need for additional information on the mode of action and metabolism of insecticides in insects and the mechanisms of the resistance to insecticides. More knowledge is also needed on the normal physiology and biochemistry of insects to permit a comparison and interpretation of the data obtained from studies on insect toxicology. Basic research in insect biochemistry and physiology, including insect nutrition, will provide a better understanding of the biochemical and physiological systems which regulate insect growth, metamorphosis, reproduction, and diapause, and the chemistry and action of the hormones which mediate these systems. Knowledge gained from such research is essential to the development of new methods of effective insect control which are safer and more selective in their action than the methods now being used. More basic information on the response of insects to light, sound, food, and sex attractants could contribute to better insect control. Insects are useful test animals for basic physiological studies on life processes.

USDA PROGRAM

The Department has a continuing long-term program involving insect physiologists, biologists and chemists engaged in basic studies in insect physiology and biochemistry and in the mode of action of insecticides and their metabolites. At the Pioneering Research Laboratory on Insect Physiology at Beltsville, Md., basic research is conducted on biochemistry and physiology of lipids in insects, insect nutrition and hormones, effect of light on insect growth and development, and effect of chemical carcinogens on insects.

The Federal scientific effort devoted to research in this area totals 10 professional man-years. Of this number 3.9 is devoted to the biochemistry and physiology of lipids in insects, 3.9 to insect nutrition and hormones, 2.0 to effect of light on insect growth and development, and 0.2 to effect of chemical carcinogens on insects.

Additional research in this area is provided by the following P. L. 480 projects: S5-ENT-3 Colombia (2 professional man-years); A7-ENT-6 India (2 professional man-years); E21-ENT-3 Poland (1 professional man-year); and E21-ENT-4 Poland (1 professional man-year).

A research contract with the Mississippi State University, No. 12-14-100-6895(33), for studies on experimentally induced insecticide resistance in a vertebrate, was executed June 24, 1963.

REPORT OF PROGRESS FOR USDA AND COOPERATIVE PROGRAMS

A. Biochemistry and Physiology of Lipids in Insects

1. Major Sterol from Cholesterol Fed American Cockroaches. In a previous study at this laboratory concerned with the fate and distribution of 4-C^{14}-cholesterol in the male American cockroach, it was found that nearly all of the radioactive sterol fraction behaved like unchanged cholesterol when analyzed by gas-liquid chromatography and reverse isotope dilution. However, other workers have reported that this insect converts 4-C^{14}-cholesterol to its "major natural sterol," a compound of unknown structure. This sterol was reported to differ from cholesterol in its chromatographic behavior in that it was slightly less polar.

To resolve these differences, the sterols (0.475 gm.) were isolated from adult male American cockroaches fed for 20 days on a synthetic diet containing 0.1% cholesterol. Analysis of the crude roach sterol by several gas-liquid chromatographic systems showed the presence of a single compound with the same relative retention time as cholesterol. Following purification through the dibromide the roach sterol and its acetate corresponded to cholesterol and its acetate in melting point and optical rotation. In addition, the optical rotatory dispersion curves, infrared and nuclear magnetic resonance spectra, and relative retention times by four gas-liquid chromatographic systems were also identical, demonstrating that unchanged cholesterol was the major sterol present in these insects. This research was conducted in cooperation with the Laboratory of Chemistry of Natural Products, National Institutes of Health, Bethesda, Md.

2. Cholesterol Turnover in Adult American Cockroach. In order to determine whether cholesterol is metabolically active or merely stored in the tissues of the American cockroach, Periplaneta americana, the rate of appearance of C^{14} labeled cholesterol in the tissues of the insect was determined over a period of 60 days. Newly emerged adult male insects were placed on a semi-defined diet containing 4-C^{14}-cholesterol. Every 10 days, representative groups of insects were selected, the alimentary tract removed from each insect, and the insects were then saponified. The unsaponifiable lipids were chromatographed on alumina and the specific activity of the tissue cholesterol was determined. The change in specific activity over the period of time was logarithmic, and when analyzed kinetically, it followed a first order reaction pattern. The half-life of the cholesterol in the tissues was calculated to be 82 days. This evidence suggests that the tissue cholesterol is constantly being renewed from the diet and that the cholesterol is being metabolized and/or excreted intact. Studies are currently being undertaken to determine if the cholesterol molecule is being modified by the insect.

3. Identity of House Fly Sterol. Preliminary studies have shown that the major sterol from unfed adult house flies reared by the CSMA procedure is not cholesterol. Other workers have reported that three sterols are present in the adults and eggs of house flies reared by the CSMA procedure. These workers designated the major sterol as "Muscasterol" and believed it to differ from cholesterol beyond C-22 on the side-chain. They further speculated that the precursor of the fly sterol could be a phytosterol which was dealkylated in the fermenting media and concentrated by the fly or dealkylated directly in the fly. However, studies in this laboratory have shown that house flies reared aseptically on a semidefined diet containing β-sitosterol (a major sterol of CSMA media) do not alter its side-chain. Thus it appeared desirable to isolate, identify, and determine the origin of the house fly sterol.

The sterols were isolated from the unsaponifiable fraction of house flies by column chromatography and digitonin precipitation. This material showed four distinct peaks when analyzed by gas-liquid chromatography (GLC). The components in order of increasing retention time were present in relative quantities of 2.8, 1.5, 74.3, and 21.4%, respectively. Neither the major sterol nor its derivatives could be separated from the fourth component (β-sitosterol) by various adsorption chromatographic systems or by crystallization from methanol.

However, a commercial source of β-sitosterol of 69% purity contained a sterol contaminant with the same relative retention time as that of the major house fly sterol. This sterol could be concentrated to 91% purity (GLC analysis) by repeated fractional recrystallization from acetone (10 times) at room temperature. Further purification through its 3,5-dinitrobenzoate yielded a compound with a final purity of 99%.

The major house fly sterol was purified under similar conditions and analyzed to be 94% pure (GLC analysis, 6% β-sitosterol). The infrared spectra of the house fly sterol and its ester derivatives were identical with that of the sterol derived from the commercial sample of β-sitosterol and its respective derivatives. The physical properties and infrared spectra of both sterols and their derivatives characterized these sterols as campesterol (a-methyl at C-24). The physical properties of campesterol and its derivatives were in complete agreement with those in the literature.

The secured identity of the house fly sterol as campesterol suggested that it was derived from the CSMA media. Yet, gas-liquid chromatography of the unsaponifiable fraction of the CSMA media showed β-sitosterol to be the major sterol, with campesterol present in a lesser amount. The possibility that the smaller size particles being consumed by the house fly were richer in campesterol was readily eliminated by experimental results which indicated that a high percentage of campesterol was present in the larger sized particles of the medium.

4. Fate of C^{14}-Cholestanol in House Fly Larvae. Cholestanol, which has been reported to serve as a sparing sterol in several insects, has also been found to fulfill this same function in the house fly, Musca domestica. To ascertain the fate of this sparing sterol in this insect, house flies were reared aseptically to pupation on a semidefined diet containing 0.2% C^{14}-cholestanol and subminimal quantities of cholesterol. Analysis of the total lipid extracts from the pupae indicated the presence of 14.5 µg. equiv. of C^{14}-sterol per insect. Column chromatographic analyses of the extracts on alumina showed that greater than 94% of the C^{14} compounds present were as free sterols and only 4.1% as sterol esters. This predominance of free sterols differs from previous findings with the German cockroach reared under sparing conditions with C^{14}-cholestanol, where about 45% of the total C^{14} compounds were found to be esterified. Further chromatographic analyses of the free sterols as their acetyl derivatives indicated a single major peak representing 99.1% of the radioactivity. In addition, two minor more polar peaks were also detected.

When an aliquot of the major peak was analyzed by reverse isotope dilution with authentic cholesterol, the radioactivity was all lost by the sixth recrystallization, indicating no conversion of cholestanol to cholesterol. Reverse isotope dilution of another aliquot of this fraction with cholestanol, by purification via the Anderson-Nabenhauer technique, and oxidation to cholestanone, indicated that greater than 97% of the radioactivity represented unchanged cholestanol. These findings were confirmed by gas-liquid chromatographic analyses. These results point to a major difference in the metabolism of cholestanol during its utilization as a sparing sterol by the house fly and the German cockroach. The latter insect converts about one-half of the cholestanol to $\Delta 7$-cholestanol, whereas in the house fly pupae nearly all of the C^{14} compounds present represented unchanged cholestanol. Studies are currently underway to determine the fate of cholestanol in the adult fly.

Results of this research have been presented in the following papers prepared for publication: Thompson, J. J., Louloudes, S. J., Robbins, W. E., Watters, J. A., Steele, J. A., and Mosettig, E. The identity of the major sterol from house flies reared by the CSMA procedure. Accepted by Jour. Insect Physiol.

Dutky, R. C., Robbins, W. E., Kaplanis, J. N., and Shortino, T. J. The sterol esters of house fly eggs. Accepted by Jour. Comp. Biochem. and Physiol.

Thompson, M. J., Dupont, J., and Robbins, W. E. The sterols of liver and carcass of 20,25-diaza-cholesterol-treated rats. Accepted by Steroids.

Robbins, W. E. Studies on the utilization, metabolism, and function of sterols in the house fly, Musca domestica. Symposium on the Use and Application of Radioisotopes and Radiation in the Control of Plant and Animal Insect Pests, sponsored by the International Atomic Energy Agency and the Food and Agriculture Organization of the United States. Accepted for publication in Proceedings.

B. Insect Nutrition and Hormones

1. Modification of Tenebrio Test for Assay of Juvenile Hormone Activity. During the course of studies on insect hormones a variation of the "Tenebrio test" for juvenile hormone activity was developed which permitted quantitation of the results, and which relied upon the retention of clearly defined pupal characters in the adult tenebrionid beetle following injection of an active material. The characters utilized were more easily and positively determined to be the result of juvenilization than the previous method of using unpigmented patches of "pupal" cuticle as the sole criterion of activity. The characters most sensitive to juvenilization were the lateral abdominal gin traps and the urogomphi. The gin traps are sclerotized structures projecting laterally from each abdominal segment and the urogomphi are sharp pointed sclerotized projections located at the end of the larval and pupal abdomens. These structures have no external counterpart in the adult except in the male beetle which retains a very small remnant of the urogomphi. These structures were retained in the adult to a greater or lesser degree depending upon the activity of the dose of the juvenilizing compound. The greatest possible effect was complete repupation and this was achieved with some of the materials investigated, such as farnesyl methyl ether which causes repupation with a dosage as low as 5 micrograms.

The outstanding effect of these compounds is the deformation of the genitalia, a situation which if serious enough would preclude copulation and hence reproduction. So far only the gross external morphological effects have been observed; investigation of effects on internal structures which may be even more sensitive is currently under study.

2. Juvenile Hormone Activity of Isoprenoid and Straight-Chain Alcohols and Derivatives. Using the above bioassay, studies on the juvenilizing activity of various isoprenoid compounds have delineated some of the structural features which influence this activity.

Reduction of farnesol to the saturated alcohol resulted in decreased but measurable activity. However, there was a complete loss of activity following removal of the hydroxyl group and reduction to the saturated hydrocarbon.

Conversion of farnesol to its methyl ether increased its activity several hundred fold, but methylation of the tertiary hydroxyl of nerolidol did not increase its activity, which emphasized the importance of the primary hydroxyl for activity. The methyl ether may be more active because of greater chemical or biochemical stability. Farnesyl acetate and farnesyl trimethyl acetate were nearly as active as the free alcohol, whereas farnesyl oleate showed little activity.

In addition to the numerous isoprenoid alcohols and their derivatives, certain straight-chain saturated alcohols from C-8 to C-14 were active, which suggests that the isoprenoid alcohols are not unique in possessing juvenile hormone activity.

Straight-chain saturated alcohols from octanol to tetradecanol exhibited low activity. With the exception of octanyl methyl ether, all of the methyl ethers possessed enhanced activity and dodecanyl and tetradecanyl methyl ethers were particularly active. The greater activity of the dodecanyl derivative is interesting in view of the fact that the carbon skeleton determining the chain length of farnesol is a 12 carbon unit. While dodecanyl methyl ether was slightly more active than farnesol, it was considerably less so than farnesyl methyl ether, suggesting that perhaps unsaturation or branching may play a major role in the degree of juvenilization. Such investigation is being pursued.

In spite of the very high order of juvenile hormone activity for farnesol and derivatives, it is unlikely that these are actually the natural hormone as they do not behave chromatographically like the active materials from Cecropia or the tobacco hornworm.

3. Juvenile and Gonadotropic Hormone Activity of Insect Extracts. Previous workers have demonstrated juvenile hormone activity in a wide variety of animals, plants, and microorganisms. This laboratory has demonstrated the gonadotropic action of an extract of the male Cecropia moth in allatectomized American cockroaches. When this extract was chromatographed on a column of silicic acid, the fraction containing the gonadotropic activity also showed juvenile hormone activity when tested by the above modification of the Tenebrio test. Rechromatography of this fraction on other adsorbents failed to separate the two activities, indicating that the hormones involved are either the same or very similar compounds.

Further investigations of crude and fractionated lipid extracts of other more common insects have revealed the presence of detectable amounts of juvenile hormone activity in a number of species. Some insects which have shown this activity are: tobacco hornworm larvae, pupae and adults, wax moth larvae, and adult males of two species of cockroaches, (Nauphoeta cinerea and Blaberus craniifer), and the silkworm moth.

Column chromatography of the crude lipids of the tobacco hornworm has shown that the juvenile and gonadotropic hormone activities are eluted together in the same fraction as the corresponding activities in Cecropia extracts. The data indicate that the hormone(s) may be the same or very similar in these two species.

4. Food Attractants and Stimulants of Alfalfa Weevil and Silkworm. Feeding attractants and stimulants have been reported to play a major role in host plant recognition and specificity in many phytophagous insects. The isolation and characterization of the attractants and stimulants would permit the role of the individual chemicals to be correlated as to their effect on the total feeding behavior of the insect, i.e., orientation, biting response, and sustained feeding.

Alfalfa weevil and silkworm larvae respond to ether extracts of alfalfa and mulberry, respectively, by oriented movements toward its source of the extracts. Long distance effects were not observed with either insect. Fractionation of the mulberry extract by column chromatography gave three different active fractions in that the silkworm larvae responded to them by orientation and/or by biting reactions. There were no reciprocal responses, indicating that the insects respond or react only to substances present in their normal host plants. Further purification of the active components is underway.

Feeding stimulants are distinguishable from feeding attractants in that they are perceived by the insect upon contact with the substrate, presumably through taste receptors located in the mouthparts. Several substances have been shown to act as feeding stimulants, particularly the simple sugars. However, feeding stimulants present in plants which presumably contain sustained feeding have been hypothesized to exert this stimulation in a synergistic manner in the presence of other stimulating substances. The adults of the alfalfa weevil will feed on an artificial diet containing water soluble substances extracted from alfalfa. They will not, however, feed on such diets in the absence of sugars. An assay technique based on this behavior of the weevil was used to extract and purify by fractionation the feeding stimulant from alfalfa. An organosoluble derivative of the stimulant has been prepared from a purified extract, which upon regeneration still is active. Work on the further purification and characterization of this stimulant is in progress. This study was conducted in cooperation with the Grain and Forage Insects Research Branch.

C. Effects of Light on Insect Growth and Development.

1. Responses of House Flies to Light. Although vitamin A has been considered unnecessary in insect diet, there is some evidence that insects have the same retinene visual pigments as mammalian rods.

House flies were reared on a vitamin A-free diet without contamination by microorganisms. These flies were compared with flies reared on the CSMA media which contained fermenting plant materials rich in carotene. Neither the aldehyde (retinene$_1$) nor the alcohol (retinol$_1$) of vitamin A was found in the heads or the eggs of flies reared on the vitamin A-free diet. Improved procedures detected both in heads of CSMA flies. This discovery of retinol in an insect supports some role for vitamin A in insect vision.

With a simple device, movement of these flies toward or from light was measured in 25 mμ increments from 300 to 725 mμ. Both diets produced photosensitive flies. Flies on the vitamin A-free diet were attracted to wavelengths from 375 to 675 mμ; CSMA flies moved away from 525, 550, and 575 mμ. The failure of flies depleted in vitamin A to be repelled by "green" light was not due to reduced intensity perception. At lowered intensity the response was slower but unchanged. Nevertheless, a thousand times more light was required for vitamin A-depleted flies to give the same optic nerve response as CSMA flies. The responses of larvae differed from adults and varied with age. Removing wings did not alter the response of adults. More females than male adults moved toward light and newly emerged flies were most photopositive. In CSMA flies, a photonegative response to 550 mμ developed two days after emergence. Experiments indicated competitive dietary effects. Something in plant material (bran, alfalfa, and yeast) when fed to larvae produced adults that were photonegative to 525-575 mμ light. β-carotene had no effect. The effects of dietary components on behavior toward light, on electroretinograms, and on histology of the eye are being investigated.

Some of the results of this study have been accepted for publication:

Cohen, Charles F. and Barker, Roy J. The vitamin A content and spectral response of house flies reared on diets with and without a vitamin A source. Accepted for publication in Jour. Cellular and Comparative Physiology.

D. Effect of Chemical Carcinogens on Insects

1. Effect of Certain Carcinogenic 2-Fluorenamine Derivatives on House Fly Larvae. Tumors, natural or induced, have been reported to occur in representatives from most of the major invertebrate phyla. In insects the best known and most intensively studied neoplasms are the hereditary tumors of Drosophila melanogaster. Although the incidence of these hereditary tumors may be increased with certain vertebrate carcinogens, attempts to chemically induce tumors in non-tumorous strains of insects have generally been unsuccessful.

To study the effect of vertebrate carcinogens on growth and development in the house fly, house fly larvae were reared aseptically on a semidefined diet containing certain representative vertebrate carcinogens. The carcinogenic compounds were coated on the dry components of the diet with a volatile organic solvent. Thirteen compounds known to produce tumors in vertebrates were tested. These included aromatic hydrocarbons, aromatic amines including four 2-fluorenamine derivatives, an azo dye, and a carbamate.

All of the 2-fluorenamine derivatives tested profoundly affected larval development when added to the diet at concentrations ranging from 0.002% to 0.04% (wet weight). In order of increasing effectiveness these compounds were: 2,7-fluorenediamine, N-2-fluorenyl-acetamide, 2-acetamide-7-fluorofluorene, and N-hydroxy-N-2-fluorenyl-acetamide. At higher concentrations these compounds were toxic to the newly hatched larvae and at intermediate and lower concentrations they were severely inhibitory to growth, development, and pupation. In addition, at certain concentrations, only a low percentage of test insects that pupated emerged as adults. Examination of last instar larvae reared on diets containing these carcinogens revealed many with gross malformations and darkened or "melanotic" areas. The pupae exhibited annular constrictions, abnormal protrusions, and imperfectly sclerotized areas.

Two structurally related compounds, fluorene and 9-fluorenone, which have been reported to be noncarcinogenic, were tested for comparison. 9-Fluorenone was nontoxic and fluorene was toxic only at the maximum concentration (0.04%). Otherwise, at the concentrations tested these two compounds neither inhibited growth, pupation, or adult emergence, nor produced the abnormalities observed with the carcinogenic 2-fluorenamine derivatives.

When a histopathological examination was made of the malformed larvae and pupae, reared on a diet containing 0.004% N-hydroxy-N-2-fluorenylacetamide, tumors were observed which were similar to hereditary tumors of Drosophila as described in the literature.

The carcinogenic action of the isomeric fluorenylamines and derivatives has been extensively studied in vertebrates and use of these compounds has greatly expanded our understanding of chemical carcinogenesis. These compounds also appear to offer a means for studying chemically induced tumors in the house fly and as such should add appreciably to knowledge of insect tumors and comparative oncology. This research was conducted in cooperation with the Insect Pathology Laboratory.

Results of this research have been presented in the following paper:

Shortino, T. J., Cantwell, G. E. and Robbins, W. E. Effect of certain carcinogenic 2-fluorenamine derivatives on house fly larvae (Musca domestica L.). Submitted to the Jour. Insect Pathology.

PUBLICATIONS REPORTING RESULTS OF USDA AND COOPERATIVE RESEARCH

Insect Resistance to Insecticides

Barker, Roy J. 1963. Comparing resistance of house flies to the eight stereoisomers of allethrin. Jour. Econ. Entomol. 56: 152-155.

Biochemistry and Physiology of Lipids in Insects

Ishii, Shoziro, Kaplanis, J. N., and Robbins, W. E. 1963. Distribution and fate of 4-C^{14}-cholesterol in the adult male American cockroach. Ann. Entomol. Soc. Amer. 56: 115-119.

Kaplanis, J. N., Monroe, R. E., Robbins, W. E., and Louloudes, S. J. 1963. The fate of dietary H^3-β-sitosterol in the adult house fly. Ann. Entomol. Soc. Amer. 56: 198-201.

Louloudes, S. J., Chambers, D. L., Moyer, D. B., and Starkey, J. H. III. 1962. The hydrocarbons of adult house flies. Ann. Entomol. Soc. Amer. 55: 442-448.

Louloudes, S. J., Shortino, T. J., and Brown, N. L. 1962. Hydroxy lecithin emulsions for treating insects. Jour. Econ. Entomol. 55: 819.

Thompson, M. J., Louloudes, S. J., Robbins, W. E., Waters, J. A., Steele, J. A., and Mosettig, E. Identity of the "house fly sterol." Biochem. & Biophys. Res. Comm. 9: 113-119.

VandenHeuvel, W. J. A., Robbins, W. E., Kaplanis, J. N., Louloudes, S. J., and Horning, E. C. 1962. The major sterol from cholesterol fed American cockroaches. Ann. Entomol. Soc. Amer. 55: 723.

Insect Nutrition and Hormones

Chen, David H., Robbins, W. E., and Monroe, R. E. 1962. The gonadotropic action of cecropia extracts in allatectomized American cockroaches. Experientia 18: 577.

Monroe, R. E., Robbins, W. E., Chambers, D. L., and Tabor, L. A. 1963. Sterol antagonists and house fly reproduction. Ann. Entomol. Soc. Amer. 56: 124-125.

Effects of Light and Sound on Insect Growth and Development

Barker, Roy J. 1963. Inhibition of diapause in *Pieris rapae* L. by brief supplementary photophases. Experientia 19: 185.

Barker, Roy J., Mayer, Ann, and Cohen, C. F. 1963. Photoperiod effects in *Pieris rapae* L. Ann. Entomol. Soc. Amer. 56: 292-294.

Cohen, Charles F., and Barker, Roy J. 1962. The relation of Vitamin A to vision in *Musca domestica* L. Amer. Zool. 2: 514 (Abstract).

Line Project Check List -- Reporting Year July 1, 1962 to June 30, 1963

Work & Line Project Number	Work and Line Project Titles	Work Locations During Past Year	Line Proj. Incl. in Summary of Progress	Line Proj. Incl. in Area & Sub-heading
ENT b1(R)	Sugarbeet insect investigations			
ENT b1-1 (R)	Control methods and biological studies of insects and mites affecting sugarbeets	Mesa, Ariz.	Yes	11-A-3 11-G-2
		Logan, Utah	Yes	11-B-2
		Twin Falls, Idaho	Yes	11-A-2 11-B-2 11-C
		Yakima, Wash.	Yes	11-A-3 11-B-2 11-G-2
ENT b2(R)	Tobacco insect investigations			
ENT b2-1	Biological control methods and biology of insects attacking tobacco foliage	Oxford, N. C.	Yes	10-A-1 10-C-1 10-D-1,2 10-E-1
		Florence, S. C.	Yes	10-A-1 10-D-1
ENT b2-2 (R)	Insecticide control methods for insects attacking tobacco foliage	Quincy, Fla.	Yes	10-B-2
ENT b2-3 (R)	Control methods and biology of soil insects that attack tobacco	Florence, S. C.	Yes	10-B-1 10-C-2
ENT b3 (R)	Greenhouse and ornamental plant insects			
ENT b3-1	Biology and methods of control of insects on greenhouse and ornamental plants	Farmingdale, N. Y.	Yes	12-B-1
		Beltsville, Md.	Yes	12-A-1 12-B-1 12-D-1
ENT b3-2	Insects in relation to diseases of greenhouse and ornamental plants	Beltsville, Md.	Yes	12-F-1
		Sumner, Wash.	No	
ENT b3-3	Biology and methods of control of insect pests of outdoor flowers	Farmingdale, N. Y.	Yes	12-A-1
		Sumner, Wash.	Yes	12-B-1
		Beltsville, Md.	Yes	12-B-1
ENT b4	Vegetable and berry insects			
ENT b4-1	Biology and methods of control of insects and mites affecting beans and peas	Twin Falls, Idaho	Yes	1-B-5
		Riverside, Calif.	Yes	1-B-7
		Beltsville, Md.	Yes	1-B-7 1-E-3
		Walla Walla, Wash.	Yes	1-A-6
		Charleston, S.C.	Yes	1-B-6
ENT b4-3 (R)	Biology and methods of control of insects affecting melons and other cucurbits	Charleston, S. C.	Yes	1-B-11

Line Project Check List -- Reporting Year July 1, 1962 to June 30, 1963

Work & Line Project Number	Work and Line Project Titles	Work Locations During Past Year	Line Proj. Incl. in Summary of Progress	Line Proj. Incl. in Area & Sub-heading
ENT b4-4 (R)	Biology and methods of control of the beet leafhopper as a pest of vegetables	Twin Falls, Idaho	Yes	1-A-5 1-B-4
		Logan, Utah	Yes	1-B-2
		Mesa, Ariz.	Yes	1-B-3
ENT b4-5	Insects in relation to diseases of vegetables and berries	Mesa, Ariz.	Yes	1-H-1,2
		Orono, Me.	Yes	2-E-1
		Yakima, Wash.	Yes	2-A-3 2-E-2
ENT b4-6 (R)	Biology, host plant relationships, and methods of control of insects that attack potato	Orono, Me.	Yes	2-A-2 2-B-2
		Yakima, Wash.	Yes	2-A-1,3,4 2-B-1
ENT b4-7 (R)	Methods of preventing deleterious residues resulting from the use of insecticides on vegetables and berries	Mesa, Ariz.	Yes	1-B-13 1-C-1
		Riverside, Calif.	Yes	1-B-13,14 1-C-1
		Charleston, S. C.	Yes	1-A-3 1-B-8,12,13 1-C-1
		Beltsville, Md.	Yes	1-B-9 1-C-1,4,5
		Logan, Utah	Yes	1-C-1
		Orono, Me.	Yes	2-C-1
		Yakima, Wash.	Yes	2-C-1
ENT b4-8 (R)	Investigations on the use of natural enemies and other biological methods for the control of vegetable and berry insects	Riverside, Calif.	Yes	1-E-4
		Beltsville, Md.	Yes	1-A-2 1-E-3,5
		Charleston, S. C.	Yes	1-A-1 1-D-1 1-E-1,2 1-G-2
		Mesa, Ariz.	Yes	1-A-1
		Orono, Me.	Yes	2-D-1
ENT b4-9 (R)	Biology and methods of control of insects and mites affecting strawberries and bramble berries	Beltsville, Md.	Yes	3-D-2
ENT-b4-10 (C)(R#2)	Biology and methods of control of insects affecting underground portions of vegetables	Charleston, S. C.	Yes	1-A-4,7,8 1-B-9,10 1-E-1,2 1-G-2 2-A-1 2-B-3
		Baton Rouge, La.	Yes	1-A-8 1-B-9

Line Project Check List -- Reporting Year July 1, 1962 to June 30, 1963

Work & Line Project Number	Work and Line Project Titles	Work Locations During Past Year	Line Proj. Incl. in Summary of Progress	Line Proj. Incl. in Area & Sub-heading
ENT b4-12 (R)	Improvement of methods and evaluation of equipment for applying insecticides to vegetable crops	Forest Grove, Oreg. Charleston, S.C.	Yes Yes	1-F-3,4 1-F-5
ENT b4-14 (R#2)	Development of methods for preventing contamination of processed vegetables and berries by field insects	Beltsville, Md.	Yes	1-A-2 1-E-5
ENT b5	Methods of treating plants and commodities regulated by plant quarantines			
ENT b5-1 (R)	Development of treatments for plants and commodities regulated by plant quarantines	Hoboken, N. J.	Yes	4-C-2 4-G-1,2, 3,4
ENT b6	Mexican fruit fly and other pests in Mexico that threaten U. S. horticulture			
ENT b6-1	Biology, ecology, habits, and natural enemies of the Mexican fruit fly and citrus black fly	Mexico City, Mex.	Yes	4-A-2 4-E-1
ENT b6-2 (R)	Studies of lures for Mexican fruit flies	Mexico City, Mex.	Yes	4-E-1
ENT b6-4	Sterilization of products infested by fruit flies in Mexico	Mexico City, Mex.	No	
ENT b7	Investigations of fruit flies in Hawaii			
ENT b7-1	Ecology and biology of fruit flies and their natural enemies in Hawaii	Honolulu, Hawaii Hilo, Hawaii Kahului, Hawaii	Yes Yes Yes	4-A-2 4-A-2 4-A-2
ENT b7-2	Development of new or improved mass production methods and manipulation techniques for fruit flies and biological control agents	Honolulu, Hawaii	Yes	4-A-2
ENT b7-3	Investigations of fruit fly lures and repellents in Hawaii	Honolulu, Hawaii Hilo, Hawaii Kahului, Hawaii	Yes Yes Yes	4-E-1 4-F 4-E-1 4-E-1
ENT b7-4	Chemical methods for control of fruit flies in Hawaii	Honolulu, Hawaii	Yes	4-E-1
ENT b7-5 (R)	Development of the sterile male release, male annihilation, and other area control and eradication procedures for fruit flies	Honolulu, Hawaii Hilo, Hawaii Kahului, Hawaii Guam	Yes Yes Yes Yes	4-E-1 4-E-1 4-E-1 4-E-1
ENT b7-6	Commodity treatments to destroy fruit flies and associated pests of quarantine importance in fresh fruits and vegetables in Hawaii	Honolulu, Hawaii	Yes	4-G-1
ENT b7-7	Ionizing radiation treatments for fruit fly infested fruits and vegetables	Honolulu, Hawaii	Yes	4-G-5

Line Project Check List -- Reporting Year July 1, 1962 to June 30, 1963

Work & Line Project Number	Work and Line Project Titles	Work Locations During Past Year	Line Proj. Incl. in Summary of Progress	Line Proj. Incl. in Area & Sub-heading
ENT b8	Deciduous fruit and nut insect investigations			
ENT b8-1 (R)	Studies of the codling moth and its control	Yakima, Wash.	Yes	3-A-1 3-B-1 3-E-1
		Kearneysville, W.Va.	Yes	3-D-1
		Vincennes, Ind.	Yes	3-A-1 3-B-1
ENT b8-2 (R)	Studies of orchard mites and their control	Yakima, Wash.	Yes	3-B-2
		Wenatchee, Wash.	Yes	3-B-2 3-E-1
		Kearneysville, W.Va.	Yes	3-A-1 3-B-2
		Vincennes, Ind.	Yes	3-B-2 3-E-1
		Wooster, Ohio	Yes	3-B-2
ENT b8-3	Studies of the plum curculio and its control	Fort Valley, Ga.	Yes	3-B-3 3-E-1
		Vincennes, Ind.	Yes	3-B-3
		Wooster, Ohio	Yes	3-B-3
		Moorestown, N. J.	Yes	3-B-3
ENT b8-4	Studies of borers attacking deciduous tree fruits and their control	Fort Valley, Ga.	Yes	3-B-4 3-E-1
		Vincennes, Ind.	Yes	3-B-4 3-E-1
ENT b8-5 (R)	Studies of the pear psylla and its control	Yakima, Wash.	Yes	3-A-1 3-B-5
		Wenatchee, Wash.	Yes	3-B-5
ENT b8-6	Studies of miscellaneous insect pests of deciduous fruits and their control	Fort Valley, Ga.	Yes	3-B-6
		Vincennes, Ind.	Yes	3-A-1 3-B-6
		Wooster, Ohio	Yes	3-A-1 3-B-6 3-E-1
ENT b8-7	Investigations of nut insects and their control	Albany, Ga.	Yes	3-A-1 3-B-7 3-E-1
		Shreveport, La.	Yes	3-B-7 3-F-1
		Vincennes, Ind.	Yes	3-B-7 3-E-1
		Wooster, Ohio	Yes	3-E-1
ENT b8-8	Grape insect investigations	Wooster, Ohio	Yes	3-B-8 3-C-1 3-F-2

Line Project Check List -- Reporting Year July 1, 1962 to June 30, 1963

Work & Line Project Number	Work and Line Project Titles	Work Locations During Past Year	Line Proj. Incl. in Summary of Progress	Line Proj. Incl. in Area & Sub-heading
ENT b9 (R)	Investigations of insect and mite vectors of deciduous tree fruit viruses			
ENT b9-1 (R)	Distribution of insects and mites in and near fruit orchards infected with virus diseases	Riverside, Calif.	Yes	3-A-2
ENT b9-2	Studies of insect vectors of phony peach virus disease and their control	Fort Valley, Ga.	Yes	3-G-1
ENT b9-3 (R)	Studies of mite vectors of peach mosaic virus disease, including biology, ecology and control	Riverside, Calif.	Yes	3-G-2
ENT b9-4 (R)	Transmission studies with possible insect and mite vectors of the latent group of stone fruit viruses	Corvallis, Oreg.	Yes	3-G-3
ENT b9-7	Transmission studies with possible insect, mite and nematode vectors of miscellaneous stone fruit virus diseases	Riverside, Calif. Fort Valley, Ga. Wenatchee, Wash. Corvallis, Oreg.	Yes Yes Yes Yes	3-G-4 3-G-4 3-G-4 3-A-2 3-G-4
ENT b9-8	Studies of possible insect and mite vectors of pear decline and their control	Riverside, Calif.	Yes	3-G-5
ENT b10	Insects of citrus and other subtropical fruits			
ENT b10-1	Biology and methods of control of citrus mites	Orlando, Fla. Riverside, Calif.	Yes Yes	4-A-1 4-B-1 4-D-1 4-A-1 4-B-1 4-D-1
ENT b10-2	Biology and methods of control of scale insects, whiteflies, and mealybugs on citrus	Orlando, Fla. Riverside, Calif. Weslaco, Tex.	Yes Yes Yes	4-B-1 4-B-1 4-E-1 4-A-1
ENT b10-3	Biology and methods of control of miscellaneous insects on citrus and other subtropical fruits	Orlando, Fla.	Yes	4-E-1
ENT b10-4 (R)	Insect vectors of tristeza and other diseases of citrus	Orlando, Fla.	Yes	4-H-1
ENT b10-5 (R)	Investigations of the biological control of citrus insects and mites	Orlando, Fla. Lake Alfred, Fla. Riverside, Calif. Weslaco, Tex.	Yes Yes Yes Yes	4-D-1 4-A-1 4-D-1 4-D-1 4-D-1

Line Project Check List -- Reporting Year July 1, 1962 to June 30, 1963

Work & Line Project Number	Work and Line Project Titles	Work Locations During Past Year	Line Proj. Incl. in Summary of Progress	Line Proj. Incl. in Area & Sub-heading
ENT b11	Japanese beetle, European chafer, and related species			
ENT b11-1	Investigations of insecticides for control of the Japanese beetle	Moorestown, N. J.	Yes	12-A-2 12-B-2 12-D-2 12-E-1
ENT b11-2	Development and improvement of treatments to permit movement of nursery stock and farm products under quarantine regulations	Moorestown, N. J.	Yes	12-G-1
ENT b11-3	Development of methods of making biological assays of insecticidal residues in soils	Moorestown, N. J.	Yes	12-B-2
ENT b11-4 (R)	Investigations of survey methods and biological and chemical control of the European chafer	Geneva, N. Y.	Yes	12-B-3 12-C-2 12-D-3 12-E-2
ENT b11-6	Ecology, biology, and natural control of the Japanese beetle	Moorestown, N. J.	Yes	12-C-1
ENT c1	Boll weevil investigations			
ENT c1-1	Ecology, life history, distribution and damage of the boll weevil	Florence, S. C. State College, Miss. Stoneville, Miss. Tallulah, La. Brownsville, Tex. Tucson, Ariz. Waco, Tex.	Yes Yes Yes Yes Yes Yes Yes	9-A-1 9-A-1 9-A-1 9-A-1 9-A-1 9-A-1 9-A-1
ENT c1-2	Development of effective and economical methods of controlling the boll weevil	Florence, S. C. State College, Miss. Stoneville, Miss. Tallulah, La. Waco, Tex. College Station, Tex.	Yes Yes Yes Yes Yes Yes	9-B-1 9-B-1 9-C-1 9-D-1 9-E-1 9-F-1 9-B-1 9-F-1 9-B-1 9-B-1 9-B-1 9-D-1
ENT c1-3	Physiological, nutritional, and biochemical research on the boll weevil	State College, Miss. Stoneville, Miss. Baton Rouge, La. Florence, S. C.	Yes Yes Yes Yes	9-A-1 9-A-1 9-A-1 9-A-1

Line Project Check List -- Reporting Year July 1, 1962 to June 30, 1963

Work & Line Project Number	Work and Line Project Titles	Work Locations During Past Year	Line Proj. Incl. in Summary of Progress	Line Proj. Incl. in Area & Sub-heading
ENT c2	Bollworm investigations			
ENT c2-1	Biology, ecology, and methods of controlling the bollworm and other lepidopterous larvae that cause a similar type of injury to cotton	College Station, Tex.	Yes	9-A-3 9-B-3
		Waco, Tex.	Yes	9-A-3 9-B-3
		Brownsville, Tex.	Yes	9-A-3 9-B-3 9-C-3 9-F-2
		Tucson, Ariz.	Yes	9-B-3 9-C-3 9-F-2
ENT c3	Cotton insects other than boll weevil, bollworm, and pink bollworm and insects attacking other fiber plants			
ENT c3-1	Biology, ecology, and methods of controlling miscellaneous insect and spider mite pests of cotton	Stoneville, Miss. Tallulah, La. College Station, Tex. Brownsville, Tex.	Yes Yes Yes Yes	9-A-3 9-B-3 9-B-3 9-A-3 9-C-3 9-D-3 9-F-3
		Waco, Tex.	Yes	9-B-3 9-C-3
		Tucson, Ariz.	Yes	9-A-3 9-B-3 9-C-3 9-F-3
ENT c4	Pink bollworm investigations			
ENT c4-1	Chemical control of the pink bollworm	Brownsville, Tex.	Yes	9-B-2
ENT c4-2	Biological control of the pink bollworm	Brownsville, Tex.	Yes	9-C-2 9-D-2
ENT c4-3	Cultural, physical and mechanical methods for control of the pink bollworm	Brownsville, Tex.	Yes	9-B-2
ENC c4-4	Plant resistance and hosts of the bollworm	Brownsville, Tex.	No	
ENT c4-5	Ecological investigations on the pink bollworm	Brownsville, Tex. Waco, Tex. Tucson, Ariz.	Yes Yes Yes	9-A-2 9-A-2 9-A-2
ENT c4-6	Physiological, biochemical and morphological research on the pink bollworm	Brownsville, Tex.	Yes	9-A-2

Line Project Check List -- Reporting Year July 1, 1962 to June 30, 1963

Work & Line Project Number	Work and Line Project Titles	Work Locations During Past Year	Line Proj. Incl. in Summary of Progress	Line Proj. Incl. in Area & Sub-heading
ENT c5	Corn insects			
ENT c5-1	Biology and ecology of the European corn borer	Ankeny, Iowa	Yes	7-A-1
		Wooster, Ohio	Yes	7-A-1
ENT c5-2	Chemical control of the European corn borer	Ankeny, Iowa	Yes	7-B-1
				7-C-1
ENT c5-3	Plant resistance to the European corn borer	Ankeny, Iowa	Yes	7-G-1
		Wooster, Ohio	Yes	7-B-1
				7-G-1
ENT c5-4	Biological control of the European corn borer	Ankeny, Iowa	Yes	7-D-1
		Wooster, Ohio	Yes	7-D-1
		Tifton, Ga.	Yes	1-B-1
				1-C-1,2
				1-F-1,2
				1-G-1
				7-A-1
				7-B-1
				7-F-1
				7-G-1
		West Lafayette, Ind.	Yes	1-G-1
				7-B-1
		State College, Miss.	Yes	7-G-1
ENT c5-6	Biology, ecology, and methods of controlling miscellaneous insects attacking corn	Stillwater, Okla.	Yes	7-A-1
		State College, Miss.	Yes	7-B-1
				7-E-1
				7-G-1
		Tifton, Ga.	Yes	7-B-1
		Brookings, S. Dak.	Yes	7-E-1
				7-F-1
				7-H-1
ENT c5-7	Plant resistance of corn to rice weevil attack	State College, Miss.	Yes	7-G-1
ENT c5-8 (R)(C)	Biology, ecology and methods of control of soil insects attacking corn	State College, Miss	Yes	7-B-1
		Brookings, S. Dak.	Yes	7-A-1
				7-F-1
				7-D-1
ENT c6	Small grain insects			
ENT c6-1	Biology, ecology and methods of control of aphids attacking small grains	Stillwater, Okla.	Yes	7-A-2
		Brookings, S. Dak.	Yes	7-B-2
				7-F-2
				7-G-2
ENT c6-2	Biology, ecology, and methods of control of mites attacking small grains	Manhattan, Kans.	Yes	7-A-2
ENT c6-3	Biology, ecology, and methods of control of hessian fly and wheat jointworm attacking small grains	Manhattan, Kans.	Yes	7-A-2
		West Lafayette, Ind.	Yes	7-A-2
				7-G-2

Line Project Check List -- Reporting Year July 1, 1962 to June 30, 1963

Work & Line Project Number	Work and Line Project Titles	Work Locations During Past Year	Line Proj. Incl. in Summary of Progress	Line Proj. Incl. in Area & Sub-heading
ENT c6-4	Biology, ecology, and methods of control of the wheat stem sawfly	Bozeman, Mont. Minot, D. Dak.	Yes Yes	7-B-2 7-G-2
ENT c6-5	Biology, ecology and methods of control of insects attacking sorghums	Stillwater, Okla.	Yes	7-A-2 7-B-2
ENT c6-6	Biology, ecology, and methods of control of soil insects and related pests of small grains	Stillwater, Okla.	Yes	7-B-2
ENT c6-7 (R)	Distribution, biology, ecology, and control of insect and mite vectors of small grain diseases	Manhattan, Kans. Baton Rouge, La.	Yes Yes	7-A-2 7-H-2 8-A 8-B 8-D 8-E
ENT c6-8	Biology, ecology, and methods of control of rice field insects	Baton Rouge, La.	Yes	8-A 8-B 8-C
ENT c7	Sugarcane insects			
ENT c7-1	Biology, ecology and methods of control of borers attacking sugarcane	Houma, La. Canal Point, Fla. Mayaguez, Puerto Rico	Yes Yes Yes	11-A-1 11-B-1 11-D-1 11-F-1 11-A-1 11-D-1 11-D-1 11-E-1
ENT c7-2	Biology, ecology, and control of insects other than borers attacking sugarcane	Houma, La. Canal Point, Fla.	Yes Yes	11-B-1 11-D-1 11-B-1 11-D-1
ENT c7-3	Biology, ecology, and methods of control of insect and mite vectors of sugarcane diseases	Houma, La.	Yes	11-G-1
ENT c8	Legume and grass insects			
ENT c8-1 (R)(C)	Biology, ecology, and control of insects attacking alfalfa and clovers	Mesa, Ariz. Tucson, Ariz. Beltsville, Md. Lincoln, Nebr. Forest Grove, Oreg. University Park, Pa.	Yes Yes Yes Yes Yes Yes	5-A-2 5-D-2 5-G-1,2, 3 5-G-1 5-A-2 5-B-2 5-D-3 5-G-6 5-A-2,3 5-B-3 5-G-1,4, 5,7,8 5-B-3 5-C-3 5-G-5 5-H-1,2

Line Project Check List -- Reporting Year July 1, 1962 to June 30, 1963

Work & Line Project Number	Work and Line Project Titles	Work Locations During Past Year	Line Proj. Incl. in Summary of Progress	Line Proj. Incl. in Area & Sub-heading
ENT c8-2	Biology, ecology, and methods of control of insects attacking legumes other than alfalfa and clovers	Columbia, Mo.	Yes	6-A-1 6-B-1 6-E-1
		Tifton, Ga.	Yes	6-B-1 6-D-1
ENT c8-3	Biology, ecology, and methods of control of insects attacking grasses	Lincoln, Nebr.	Yes	5-A-4 5-D-4
		Tifton, Ga.	Yes	5-A-4 5-B-4
ENT c8-4	Insect vectors of pathogenic agents affecting legumes and grasses	University Park, Pa.	Yes	5-H-1,2
		Tifton, Ga.	Yes	5-H-3
		Columbia, Mo.	Yes	6-F-1
ENT c8-5	Insecticide residues on forage crops	Tifton, Ga.	Yes	5-C-1,2
		Forest Grove, Oreg.	Yes	5-C-3
ENT c9	General feeder insects			
ENT c9-1	Biology, ecology, and biological methods of control of armyworms and cutworms	Baton Rouge, La.	Yes	5-D-5
ENT c9-2	Biology, ecology, and methods of control of grasshoppers	Mesa, Ariz.	Yes	5-A-1
		Bozeman, Mont.	Yes	5-A-1 5-B-1 5-D-1
		Columbia, Mo.	Yes	5-D-1
ENT c9-3	Biology, ecology, and methods of control of white-fringed beetles	Florala, Ala.	Yes	5-A-5 5-B-5 5-E-1 5-F-1
ENT c10	Bee culture investigations (including other pollinating insects)			
ENT c10-1 (C)	Biology of diseases and pests of honey bees and development of control methods	Beltsville, Md.	Yes	18-B-1,2,3,4,8
		Baton Rouge, La.	Yes	18-B-5
		Laramie, Wyo.	Yes	18-B-6,7,8,9,10,11,12,13,14
		Madison, Wis.	Yes	18-B-6,8,9,12
ENT c10-2	Biology, breeding, and management for improvement in productivity of honey bees	Baton Rouge, La.	Yes	18-A-1,4
		Madison, Wis.	Yes	18-A-2,3,4,5,6,7,8
		Tucson, Ariz.	Yes	18-A-5

Line Project Check List -- Reporting Year July 1, 1962 to June 30, 1963

Work & Line Project Number	Work and Line Project Titles	Work Locations During Past Year	Line Proj. Incl. in Summary of Progress	Line Proj. Incl. in Area & Sub-heading
ENT c10-3	Behavior and utilization of honey bees in the pollination of agricultural crops	Madison, Wis.	Yes	18-C
		Baton Rouge, La.	Yes	18-C
		Logan, Utah	Yes	18-C
		Beltsville, Md.	Yes	18-C
ENT c10-4	Biology and utilization of insects other than honey bees in the pollination of agricultural crops	Logan, Utah	Yes	18-D-5,6,8
		Madison, Wis.	Yes	18-D-1
		Baton Rouge, La.	Yes	18-D-1,2
		Tucson, Ariz.	Yes	18-D-3,4,7
ENT c10-5	Effects of pesticides and other farm practices on honey bees and other pollinating insects	Tucson, Ariz.	Yes	18-E
		Beltsville, Md.	Yes	18-E
		Logan, Utah	Yes	18-E
ENT h1	Mosquitoes, sand flies, and gnats investigations			
ENT h1-1 (R#2)	Development of more effective insecticides and other materials and methods for controlling mosquitoes	Gainesville, Fla.*	Yes	13-B-1 14-B-1 16-B-2 17-B-1 17-D-1
		Corvallis, Oreg.	Yes	13-B-1 13-E-1 14-B-1 14-E-1 16-A-2 16-B-2 16-D-2 17-A-1 17-D-1
		Fresno, Calif.	Yes	13-D-1 14-D-1 16-A-2 17-B-1 17-C-1
ENT h1-4 (R#2)	Studies on the distribution, abundance, taxonomy, and biology of mosquitoes affecting agriculture	Kerrville, Tex.	Yes	13-A-1 14-A-1
		Corvallis, Oreg.	Yes	13-A-1 14-A-1 16-A-2 16-D-2 17-A-1
		Fresno, Calif.	Yes	13-A-1 14-A-1 16-A-2 17-A-1
		Gainesville, Fla.*	Yes	17-A-1
	*Research at Gainesville, Fla. transferred from Orlando, Fla., in June 1963.			

Line Project Check List -- Reporting Year July 1, 1962 to June 30, 1963

Work & Line Project Number	Work and Line Project Titles	Work Locations During Past Year	Line Proj. Incl. in Summary of Progress	Line Proj. Incl. in Area & Sub-heading
ENT h1-5	Development of repellents and other materials and methods to protect man and animals from mosquitoes, sand flies and gnats	Gainesville, Fla.*	Yes	17-A-1 17-D-1
ENT h1-15 (R)	Studies on the relationship of water and land management procedures to mosquito breeding in water impoundments and in irrigated farming areas	Fresno, Calif.	Yes	16-A-2
ENT h1-16 (R#2)	Studies on the biology and control of black flies, sand flies, and other gnats and their relationship to disease transmission, especially on livestock and poultry	Corvallis, Oreg. Denver, Colo. Gainesville, Fla.*	Yes Yes Yes	15-A-3 15-E-1 17-A-3 17-D-4
ENT h2	Investigations of flies affecting man and livestock			
ENT h2-1 (R#2)	Development of insecticides, repellents, and other materials and methods for the control of horn flies, stable flies, and face flies	Kerrville, Tex.	Yes	13-A-2 13-A-4 13-B-2 13-B-4 13-E-2 13-E-4 14-A-2 14-A-4 14-B-2 14-B-4 14-E-2 14-E-4
		Corvallis, Oreg.	Yes	13-A-2 13-B-4 13-E-4 14-A-2 14-B-4 14-E-4
		Lincoln, Nebr.	Yes	13-A-3 13-B-2 13-B-3 13-B-4 13-D-2 13-D-3 13-E-3 14-A-3 14-B-2 14-B-3 14-B-4 14-D-2 14-D-3 14-E-3
		Stoneville, Miss.	Yes	13-B-4 14-B-4
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Line Project Check List -- Reporting Year July 1, 1962 to June 30, 1963

Work & Line Project Number	Work and Line Project Titles	Work Locations During Past Year	Line Proj. Incl. in Summary of Progress	Line Proj. Incl. in Area & Sub-heading
ENT h2-5 (R##2)	Development of improved larvicides and other materials and methods for the control of screw-worms and fleeceworms	Mission, Tex.**	Yes	15-A-1 15-D-1
ENT h2-7 (R##2)	Studies of irradiation and radioactive insecticides on flies and other arthropods affecting man and animals	Corvallis, Oreg.	Yes	13-A-1 13-A-6 13-E-6 14-A-1 14-A-6 14-E-6 16-A-2
		Gainesville, Fla.*	Yes	13-E-6 14-E-6 17-D-1,2
		Mission, Tex.**	Yes	13-A-5 13-E-5 14-A-5 14-E-5 15-A-1
		Kerrville, Tex.	Yes	13-E-2,7,8 14-E-2,7,8 15-D-1,2
ENT h2-9 (R##2)	Development of insecticides, attractants, and other materials and methods for the control of house flies and blow flies	Gainesville, Fla.*	Yes	13-A-6 13-B-6 14-A-6 14-B-6 16-B-1 16-D-1 17-A-2 17-B-2 17-D-2
		Corvallis, Oreg.	Yes	13-A-6 13-B-6 13-E-6 14-A-6 14-B-6 14-E-6 16-A-1 16-B-1 16-D-1 17-A-2 17-B-2 17-D-2
		Lincoln, Nebr.	Yes	13-D-2 14-D-2

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**Satellite laboratory of Kerrville, Tex. established in August, 1962.

Line Project Check List -- Reporting Year July 1, 1962 to June 30, 1963

Work & Line Project Number	Work and Line Project Titles	Work Locations During Past Year	Line Proj. Incl. in Summary of Progress	Line Proj. Incl. in Area & Sub-heading
ENT h2-11 (R#2)	Studies on the biology and control of horse flies and deer flies as they relate to pests of animals and vectors of disease	Stoneville, Miss.	Yes	13-A-8 13-B-8 14-A-8 14-B-8
		Corvallis, Oreg.	Yes	15-A-3
ENT h2-14 (R)	Development of repellents and other methods to protect man from horse flies, deer flies, and stable flies		No	
ENT h2-15 (R)	Development of improved media and mass rearing and distribution techniques for screw-worm control	Mission, Tex.*	Yes	13-A-5 14-A-5 15-A-1
ENT h2-16 (R)	Development of attractants and other materials and methods for estimating and controlling natural screw-worm populations	Mission, Tex.*	Yes	15-A-1 15-D-1
ENT h2-17	Development of physical and mechanical methods of controlling flies and other pests of livestock	Beltsville, Md.	Yes	13-F-1 14-F-1 16-D-1
ENT h3(R)	Cattle grub and bot fly investigations			
ENT h3-1 (R#2)	Development of new insecticides and other materials and methods for the control of grubs and bots affecting livestock	Kerrville, Tex.	Yes	13-A-7 13-B-7 14-A-7 14-B-7 15-B-1
		Corvallis, Oreg.	Yes	13-A-7 13-B-7 14-A-7 14-B-7
ENT h4	Lice, mites, ticks, and fleas affecting man and animals investigations			
ENT h4-1 (R#2)	Development of improved insecticides and other materials and methods for the control of lice affecting livestock	Kerrville, Tex.	Yes	13-A-9 14-A-9 15-A-2 15-B-2
		Stoneville, Miss.	Yes	13-B-9 14-B-9
		Corvallis, Oreg.	Yes	15-B-2
ENT h4-3 (R#2)	Development of improved materials and methods for the control of external parasites of poultry	Corvallis, Oreg. Kerrville, Tex. Stoneville, Miss.	Yes Yes Yes	16-B-3 16-B-3,4 16-B-3
ENT h4-7 (R)	Development of insecticides and other methods for the control of human lice and itch mites affecting man	Gainesville, Fla.**	Yes	17-B-4 17-D-5

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Line Project Check List -- Reporting Year July 1, 1962 to June 30, 1963

Work & Line Project Number	Work and Line Project Titles	Work Locations During Past Year	Line Proj. Incl. in Summary of Progress	Line Proj. Incl. in Area & Sub-heading
ENT h4-8 (R)	Development of insecticides and other methods for the area control of ticks, mites, and fleas with particular reference to protecting man	Gainesville, Fla.*	Yes	17-B-5
ENT h4-9 (R)	Development of repellents and other methods to protect man from mites, ticks, and fleas	Gainesville, Fla.*	Yes	17-D-6
ENT h4-10 (R)	Development of insecticides and other materials and methods for the control of ticks and sheep ked on animals	Kerrville, Tex.	Yes	13-A-10 13-B-10 13-F-1 14-A-10 14-B-10 14-F-1 15-B-3 15-D-2
		Corvallis, Oreg.	Yes	15-B-3
ENT h4-11	Studies on the role of lice, mites, ticks, fleas, and other arthropods in the transmission of anaplasmosis and other diseases of livestock	Kerrville, Tex.	Yes	13-G-1 14-G-1
		Stoneville, Miss.	Yes	13-G-1 14-G-1
		Corvallis, Oreg.	Yes	13-G-1 14-G-1
		Beltsville, Md.	Yes	13-G-1 14-G-1
ENT h7	Toxicity and residue studies on insecticides and repellents in relation to the control of insects affecting livestock			
ENT h7-1 (R#2)	Investigations relating to the acute and chronic toxicity of insecticides, repellents, and other materials to livestock	Kerrville, Tex.	Yes	13-C-2 14-C-2 15-C-1 16-C-1
ENT h7-2 (R)	Extent of storage of insecticides in animal tissues and amount secreted in milk of dairy cattle when used for insect control	Kerrville, Tex.	Yes	13-C-1 14-C-1
ENT h7-4 (R)	Development of quantitative bioassay methods for analysis of insecticidal chemical residues	Kerrville, Tex.	No	
ENT h10	Household insect investigations			
ENT h10-1 (R)	Development of measures for the control of insects in homes	Gainesville, Fla.*	Yes	17-A-4 17-B-3,6, 7 17-D-3

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Line Project Check List -- Reporting Year July 1, 1962 to June 30, 1963

Work & Line Project Number	Work and Line Project Titles	Work Locations During Past Year	Line Proj. Incl. in Summary of Progress	Line Proj. Incl. in Area & Sub-heading
ENT j1	Identification and classification of insects			
ENT j1-1	Identification and classification of hemipterous insects	Washington, D. C.	Yes	20-A-1,4 20-B
ENT j1-2	Identification and classification of beetles	Washington, D. C.	Yes	20-B 20-C-4
ENT j1-3	Identification and classification of moths and butterflies	Washington, D. C.	Yes	20-B 20-C-2
ENT j1-4	Identification and classification of grasshoppers and allied insects	Washington, D. C.	Yes	20-B 20-C-5,6
ENT j1-5	Identification and classification of two-winged flies	Washington, D. C.	Yes	20-A-2 20-B 20-C-3
ENT j1-6	Identification and classification of thrips	Washington, D. C.	Yes	20-B
ENT j1-7	Identification and classification of hymenopterous insects	Washington, D. C.	Yes	20-A-3 20-B
ENT j1-8	Identification and classification of mites, chiggers and ticks	Washington, D. C.	Yes	20-B 20-C-1
	(Diptera catalogue - not included in a line project)	Washington, D. C.	Yes	20-A-5
ENT j2	Utilization of insect enemies in the control of insect pests and weeds			
ENT j2-4	Receipt and distribution of foreign insect enemies of insect pests and weeds	Berkeley, Calif. Moorestown, N. J. Riverside, Calif.	Yes Yes Yes	21-D-8 21-D-1,2, 3,4,5,6, 7,9,10 21-D-1,4
ENT j2-6	Biological control of weeds	Berkeley, Calif. Buenos Aires, Argentina Rabat, Morocco* Rome, Italy	Yes Yes Yes Yes	21-C-11, 12,13 21-C-8 21-C-10 21-C-9
ENT j2-7 (R)	Search for and importation of foreign parasites and predators of insect pests	Moorestown, N. J. Riverside, Calif. Paris, France	Yes Yes Yes	21-A-1 21-C-1,7 21-A-1 21-C-7 21-A-1 21-C-2,3, 4,5,6,7, 9
ENT j2-8 (R)	Search for and importation of foreign insect enemies of weeds *Station closed in March, 1963.	Buenos Aires, Argentina Rabat, Morocco* Rome, Italy	Yes Yes Yes	21-C-8 21-C-9 21-B-1

Line Project Check List -- Reporting Year July 1, 1962 to June 30, 1963

Work & Line Project Number	Work and Line Project Titles	Work Locations During Past Year	Line Proj. Incl. in Summary of Progress	Line Proj. Incl. in Area & Sub-heading
ENT m1	Chemical investigations of products of natural origin for insect control			
ENT m1-14	Investigation of plants as sources of insecticides, synergists, insect repellents or attractants, or insect antimetabolites	Beltsville, Md. State College, Miss.	Yes Yes	19-A-2 19-A-2
ENT m1-15	Investigation of substances naturally occurring in insects that might be used to upset their development or reproduction or otherwise affect their vital processes	Beltsville, Md.	Yes	19-A-1
ENT m2	Chemical investigations to develop synthetic organic materials for insect control			
ENT m2-1 (R)	Preparation of synthetic organic compounds for testing as insecticides, and synergists	Beltsville, Md. Orlando, Fla.	Yes Yes	19-B-1 19-B-1
ENT m2-4 (R)	Development of chemical formulations for insect control	Beltsville, Md. Orlando, Fla.	Yes Yes	19-B-2 19-B-2
ENT m2-13	Chemical investigations of radioactively labeled insecticides	Beltsville, Md. Orlando, Fla.	Yes Yes	19-B-1 19-B-1
ENT m2-15	Preparation of synthetic organic compounds for testing as insect control or eradication agents through effects other than death	Beltsville, Md. Orlando, Fla. State College, Miss.	Yes Yes Yes	19-B-1 19-B-1 19-B-1
ENT m3	Analysis of pesticides, pesticide residues, and accessory materials			
ENT m3-5 (R)	Analysis of insect control chemicals, their formulations, and accessory materials	Beltsville, Md.	Yes	19-B-1,2 19-C
ENT m3-6*	Determination of residues of insect control chemicals in plant and animal products and in soils	Beltsville, Md. Tifton, Ga. Vincennes, Ind. Moorestown, N. J.** Kerrville, Tex. Yakima, Wash.	Yes Yes Yes Yes Yes Yes	1-C-1,4, 5 2-C-1 3-C-1 9-C-1 10-C-1 13-C-1 1-C-1,2 5-C-1,2 6-C-1 7-C-1 3-C-1 7-C-1 12-B-2 13-C-1 1-C-1,3 2-C-1,2, 3 3-B-1 3-C-1 5-C-3

*Supersedes ENT m3-3(R)
**Station closed in December 1962.

Line Project Check List -- Reporting Year July 1, 1962 to June 30, 1963

Work & Line Project Number	Work and Line Project Titles	Work Locations During Past Year	Line Proj. Incl. in Summary of Progress	Line Proj. Incl. in Area & Sub-heading
ENT m4	Chemical investigations on fumigants and aerosols for control of insect pests			
ENT m4-1 (R)	Development of formulas and dispensing equipment for aerosols to control insects	Beltsville, Md.	Yes	19-D
ENT m9	Laboratory tests to determine the effectiveness of insect control materials			
ENT m9-1 (R#2)	Comparison of the toxic, attractant, arrestant, and repellent action of chemical materials to test insects	Beltsville, Md. Brownsville, Tex.	Yes Yes	19-E-1 19-E-1
ENT m9-3 (R)	Comparison of insecticidal materials in gas-propelled aerosols and space sprays	Beltsville, Md.	Yes	19-E-3
ENT m9-4	Biological evaluation of materials for insect control through effects other than death	Beltsville, Md. Brownsville, Tex.	Yes Yes	19-E-2 19-E-2
ENT m10	Methods for disinsectization of aircraft (Not divided into line projects)	Beltsville, Md.	Yes	19-F
ENT m11	Development of methods of analysis for pesticides and pesticide residues			
ENT m11-2 (R)	Development of methods of analysis for insect control chemicals	Beltsville, Md. Orlando, Fla. Tifton, Ga. Vincennes, Ind. Moorestown, N. J.* Kerrville, Tex. Yakima, Wash.	Yes Yes Yes Yes Yes Yes Yes	19-C 19-C 19-C 19-C 19-C 19-C 19-C
ENT P 1	Insect Pathology Laboratory	Beltsville, Md.	Yes	22-A-1,2,3,4,5 22-B-1,2,3,4,5,6 22-C-1 22-D-1,2,3,4,5
ENT P 2	Insect Physiology Laboratory	Beltsville, Md.	Yes	23-A-1,2,3,4 23-B-1,2 3,4 23-C-1 23-D-1

*Station closed in December 1962.

Line Project Check List -- Reporting Year July 1, 1962 to June 30, 1963

Work & Line Project Number	Work and Line Project Titles	Work Locations During Past Year	Line Proj. Incl. in Summary of Progress	Line Proj. Incl. in Area & Sub-heading
	P. L. 480 Projects			
A7-ENT-1	Investigations of parasites, predators, and pathogens of sugarcane borers in India	India	Yes	11-D-1 21-D-9
A7-ENT-2	Survey of beneficial parasites and predators of agricultural and horticultural crops in the Indian Union	India	No	21
A7-ENT-5	Investigations of parasites, predators, and pathogens of insect pests of paddy (rice)	India	No	21
A7-ENT-6	Nutritional studies on the silkworm Bombyx mori L. - its requirements for vitamins and amino acids and its nutrition in relation to the mineral nutrition of its host plant, mulberry (Morus indica) and studies on the host specificity of the silkworm Bombyx mori L.	India	No	23
A7-ENT-7	Survey for natural enemies of witchweed, and of water hyacinth and other weeds affecting waterways, in India	India	No	21
A7-ENT-8	Developing methods for large-scale rearing of parasites under laboratory conditions	India	No	21
A7-ENT-9	Investigations of parasites, predators, and pathogens of the European corn borer and Heliothis spp. in India	India	Yes	21-D-9
A7-ENT-10	Acarine disease problem of honey bees	India	Yes	18-B-4
A7-ENT-17	Control of the coconut rhinoceros beetle Oryctes rhinoceros L.	India	No	21
A7-ENT-20	Studies on microbiology and pathology of insect pests of crop plants	India	No	22
A7-ENT-24	Systematic and biological studies of Indian thrips	India	No	20
A7-ENT-25	Research on insect pests of maize with special reference to stalk borers	India	No	7
A10-ENT-5	Host plant-vector and host plant-virus relationships of the rough dwarf virus of corn and methods for control of the disease	Israel	Yes	7-H-1
A17-ENT-5	Studies on indigenous natural enemies of scale insects and fruit flies	Pakistan	Yes	3-D-1 4-D-1 21
A17-ENT-7	Investigations on the natural enemies of corn borers	Pakistan	No	21
A17-ENT-8	Studies on the natural enemies of insect pests of rice	Pakistan	No	21

Line Project Check List -- Reporting Year July 1, 1962 to June 30, 1963

Work & Line Project Number	Work and Line Project Titles	Work Locations During Past Year	Line Proj. Incl. in Summary of Progress	Line Proj. Incl. in Area & Sub-heading
A17-ENT-9	Studies on the insect enemies of noxious weeds in Pakistan	Pakistan	No	21
A17-ENT-10	Studies on oriental leafhoppers (Typhlocybinae)	Pakistan	No	20
E8-ENT-1	An investigation of the population dynamics of Calligypona pellucida (F.) and of the nature of the injury to oats and spring wheat caused by this plant hopper	Finland	Yes	7-H-2
E15-ENT-1	A study of Acarine disease of honey bees	Italy	Yes	18-B-4
E21-ENT-2	Studies on the possibility of biological control of aphids and scale insects and the effect of pesticides on the natural enemies of these pests	Poland	Yes	3-D-1 21
E21-ENT-3	The influence of some vitamins on the physiology of the Colorado potato beetle (Leptinotarsa decemlineata Say)	Poland	No	23
E21-ENT-4	The causes and the role of diapause of insect pests	Poland	No	23
E21-ENT-5	Studies on the differences in susceptibility of spider mites to acaricides and on cholinesterases in spider mites as influenced by acaricides	Poland	Yes	3-B-2
E21-ENT-6	The nature of infectious processes caused by protozoa in insects	Poland	No	22
E21-ENT-7	The development, maturation, and production of drones and the natural mating of virgin and drone honey bees	Poland	Yes	18-A-1
E21-ENT-9	Insect vectors of virus diseases of various forage legumes	Poland	Yes	5-G-4 5-H-4
F4-ENT-2	Survey of the insect fauna of Egypt	Egypt	No	20
F4-ENT-3	Induced sterility in males of the Mediterranean fruit fly, Ceratitis capitata, as a means of controlling and possibly eradicating that pest	Egypt	Yes	4-E-1
S3-ENT-1	Biology and breeding of honey bees	Brazil	Yes	18-A-1
S3-ENT-7	Catalogue of insects living on plants in Brazil and of the parasites and predators of the insects	Brazil	No	21
S5-ENT-2	A biochemical study of Drosophila (vinegar flies) classification	Colombia	No	20
S5-ENT-3	The metabolism of temperature acclimated Drosophila	Colombia	No	23
S9-ENT-1	Studies of the parasites and predators of several insects of economic importance	Uruguay	No	21

Line Project Check List -- Reporting Year July 1, 1962 to June 30, 1963

Work & Line Project Number	Work and Line Project Titles	Work Locations During Past Year	Line Proj. Incl. in Summary of Progress	Line Proj. Incl. in Area & Sub-heading
S9-ENT-3	Investigations on the biology and biological control of the fire ant, Solenopsis saevissima richteri, in Uruguay	Uruguay	No	17
S9-ENT-6	Systematic collections, identification, and classification of the grasshoppers of Uruguay and neighboring territories of Southern Brazil, Southern Paraguay, and adjacent provinces of Argentina	Uruguay	Yes	20-C-6